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INHERITANCE OF RESISTANCE TO LOOSE AND COVERED SMUTS IN MARKTON OAT HYBRIDS[1]

By GEORGE M. REED, *curator, Brooklyn Botanic Garden,* and T. R. STANTON, *senior agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Inheritance of smut resistance in oat hybrids has attracted the attention of an increasing number of investigators. The literature has been reviewed by Coffman et al. (*2*)[2] Reed (*6*), and Johnson (*4*). Since these reviews were published other papers have appeared by Nicolaisen (*5*), Schattenberg (*9*), Stanton, Coffman, and Tapke (*10*), Stanton, Reed, and Coffman (*11*), Reed (*7, 8*), and Austin and Robertson (*1*).

The present paper is concerned with the investigation of the inheritance of resistance to loose smut (*Ustilago avenae*) (Pers. Jens.) and covered smut (*U. levis* (Kell. and Sw.) Magn.) in oat hybrids involving the smut-resistant Markton and five other parental varieties showing contrasting reactions to these smuts.

MATERIAL AND METHODS

The strains of the varieties used in these hybrids belong to *Avena sativa* L. and have been grown by the senior author for many years in connection with his investigations of the oat smuts. With the exception of Markton, all these type varieties have been described by Etheridge (*3*). Information on the origin and characters of Markton is given in other publications (*2, 10, 11*). The hybrids were made primarily for studies of the mode of inheritance of smut resistance and not for the development of resistant varieties for commercial production. In all hybrid combinations the female parent is named first. On the basis of the smut reaction of these varieties the different hybrids may be grouped as follows:

Group 1.—One parental variety resistant and the other susceptible to both loose and covered smut:
Hybrid 50, Canadian × Markton.
Hybrid 51, Early Champion × Markton.
Hybrid 52, Markton × Early Champion.
Hybrid 53, Victor × Markton.

Group 2.—One parental variety resistant to both smuts and the other susceptible to loose smut but resistant to covered smut:
Hybrid 56, Gothland × Markton.

Group 3.—One parental variety resistant to both smuts and the other susceptible to covered smut, but resistant to loose smut, the direct opposite or antithesis of hybrid 56:
Hybrid 60, Monarch × Markton.

The hybrids were made in 1928 at Aberdeen, Idaho, by Clem Ault, under the direction of G. A. Wiebe, who at that time was assistant

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[2] Reference is made by number (italic) to Literature Cited, p. 176.

agronomist in charge of the cooperative cereal experiments at the Aberdeen substation. Seed of the special strains was furnished by the senior author and forwarded to Aberdeen by the junior author for sowing.

The F_1 plants were grown at the Brooklyn Botanic Garden in 1929, and the F_2, F_3, and F_4 in subsequent years. The method of inoculation employed was developed by the senior author in the course of his investigations with the oat smuts. After the removal of the hulls, the caryopses were inoculated with the dry spores of *Ustilago avenae*-Missouri or *U. levis*-Missouri as described. The seeds were germinated at a temperature of 20° C. in sand containing about 20 percent moisture. The seedlings usually emerged in 4 days and were large enough to transplant 2 to 3 days later.

In the case of all hybrids, three series of F_2 plants were grown: (1) Uninoculated, (2) inoculated with loose smut, and (3) inoculated with covered smut. Consequently, in growing the F_3 generations, there were three groups of progenies available: (1) Those descended from uninoculated F_2 plants, (2) those descended from F_2 plants inoculated with loose smut, and (3) those descended from F_2 plants inoculated with covered smut.

EXPERIMENTAL DATA

REACTION OF PARENTAL VARIETIES

The reaction of the parental varieties to the Missouri races of loose smut and covered smut was determined. During the period from 1930 to 1934, populations of the parents were grown along with the various hybrid generations, and all the data for the parental varieties and data obtained in 1930 on the reactions of the F_2 plants are summarized in table 1.

TABLE 1.—*Reaction to the Missouri races of loose and covered smut of inoculated F_2 plants of 5 oat hybrid combinations and their parental varieties grown at the Brooklyn Botanic Garden, 1930* [1]

Group, parental variety, and hybrid	Hybrid no.	Reaction to *Ustilago avenae*			Reaction to *Ustilago levis*		
		Plants grown	Plants infected		Plants grown	Plants infected	
Group 1:		*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
Canadian (119) [2]		319	316	99.1	373	370	99.2
Canadian × Markton	50	81	7	8.6	80	26	32.5
Markton (752)		681	9	1.3	763	7	.9
Early Champion (150)		320	290	90.6	314	267	85.0
Early Champion × Markton	51	230	26	11.3	227	66	29.1
Markton × Early Champion	52	75	12	16.0	20	4	20.0
Victor (126)		509	488	95.9	690	656	95.1
Victor × Markton	53	182	22	12.1	130	30	23.1
Group 2:							
Gothland (152)		672	645	96.0	751	0	0
Gothland × Markton	56	170	29	17.1	161	9	5.6
Group 3:							
Monarch (161)		1,079	4	.4	1,078	1,036	96.1
Monarch × Markton	60	158	2	1.3	246	54	22.0

[1] All data for parental varieties for 1930–34 included.

[2] Numbers in parentheses indicate special seed numbers used by G. M. Reed to designate his particular experimental strains of these varietal types.

The parent varieties Canadian, Early Champion, Gothland, and Victor gave from 90.6 to 99.1 percent infection with loose smut. Only 4 of 1,079 plants grown of the resistant Monarch were infected with *Ustilago avenae*. Canadian, Early Champion, Monarch, and Victor were very susceptible to covered smut, giving from 85 to 99.2 percent infection. No smutted plants appeared in the resistant Gothland.

Markton showed a very high degree of resistance to both loose and covered smuts. Most of the populations grown contained no smutted individuals. Occasionally, however, a smutted plant was observed. This occurred somewhat more frequently with loose smut than with covered smut.

Among the plants grown from inoculated seed of Markton, a few were observed that were described as blasted.[3] Such plants usually produced infertile spikelets. When these plants were first noted, the sterile condition was not attributed to possible infection by the smut organism. The fact, however, that such types of panicles appeared rather commonly among the hybrids of which Markton was one of the parents suggested that this sterility was really due to the infection of the plant by the smut. A study of these plants revealed the fact that various degrees of sterility appeared in the panicles. In some cases the spikelets were mere rudiments (fig. 1); in others they were better developed and frequently contained a small number of smut spores (fig. 2). Typically smutted panicles (fig. 3) were observed in the same rows.

This peculiar behavior of Markton toward the smuts must be considered in the study of the inheritance of resistance. For the most part plants in which smut spores could not be readily observed were not counted as infected. This was especially true in the earlier phases of the investigation. If this type of infected plant had been included in recording the data, the percentage of smut in the F_2, F_3, and later generations would be somewhat higher.

REACTION OF THE F_2 GENERATION

The data obtained on the F_2 generation for all six crosses are recorded in table 1.

While Canadian has shown equally high susceptibility to both smuts, in the Canadian × Markton combination (hybrid 50) a much higher percentage of the plants of the hybrids were infected with covered smut than with loose smut.

In hybrid 51, in which Early Champion was used as the female parent and Markton as the male, and in hybrid 52, its reciprocal, it will be noted from table 1 that considerably higher percentages were obtained with covered smut than with loose smut and that there was some variation in the reaction in F_2 of the populations from the initial and reciprocal crosses.

In hybrid 53, Victor × Markton, a much higher percentage of infection also was obtained with the covered smut, although the Victor parent was almost 100 percent infected with both smuts.

[3] A relatively small percentage of similar sterile plants were observed as early as 1925 in F_2 populations of Markton × Idamine, Markton × Victory, etc., at the Aberdeen substation, by T. R. Stanton, F. A. Coffman, and G. A. Wiebe. While no systemic examination was made at the time, it was believed that the blasted appearance of the plants resulted from a form of sterility probably caused by smut infection.

FIGURE 1.—Rudimentary panicles and spikelets occurring on Markton hybrid 50-c-F-229-2, showing characteristic sterility and no external evidence of smut pustules.

FIGURE 2.—Rudimentary panicles and spikelets occurring on Markton hybrid 51–F_6–223–3, representing a second type of sterility with some smut pustules but on undersized kernels.

FIGURE 3.—Typical development of *Ustilago levis* on Markton hybrid 51-F_4-223-3.

Hybrid 56, Gothland × Markton, represents the second group of oat hybrids included in this study, in which one parent, Markton, is resistant to both smuts, while the other parent, Gothland, is susceptible to the loose smut but resistant to the covered. It is interesting to note that a considerable number of F_2 plants were infected by covered smut, although both Gothland and Markton, as shown by the data in table 1, are extremely resistant.

Hybrid 60, Monarch × Markton, represents the third group of hybrids, in which a variety (Markton) resistant to both smuts is crossed with another variety (Monarch) susceptible to the covered smut but resistant to the loose smut. In this combination Monarch was used as the female parent. The average infection for all F_2 plants was 22 percent for covered smut. Of the F_2 plants inoculated with loose smut, 1.3 percent were unexpectedly infected.

The behavior of hybrid 60 in the loose smut experiments is similar to that of hybrid 56 in the covered smut experiments. The appearance of plants infected with *Ustilago avenae* in F_2 populations of hybrid 60, in which both parents have shown virtually 100 percent resistance, is of much genetic interest. In hybrid 56 the appearance of 5.6 percent of smutted plants in the *U. levis* series with both parents 100-percent resistant is likewise unusual.

REACTION OF THE F_3 GENERATION

A summary of data on the reaction of the F_3 progenies of the different hybrids is given in table 2. The progenies are grouped in 11 classes on the basis of class centers for percentages of smutted plants. On the basis of behavior, the F_2 progenies are grouped empirically as resistant (no infected plants observed), segregating (50 percent infection or less), and susceptible (more than 50 percent infection).

TABLE 2.—*Distribution of F_3 progenies of hybrids between Markton and 5 varieties of oats, based on the percentage of plants infected with Ustilago avenae and U. levis*

REACTION TO U. AVENAE

Smut used for inoculation in F_2 and hybrid	Hybrid no.	Infection in F_2 [1]	F_3 progenies in indicated class center for percentage of infection [2]													Total F_3 progenies	F_3 progenies infected
			Resistant	Segregating						Susceptible							
			0	5	15	25	35	45	Total	55	65	75	85	100	Total		
		Percent	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
Not inoculated:																	
Canadian × Markton	50	-------	25	14	10	2	4	2	32	1	0	0	0	0	1	58	56.9
Early Champion × Markton [3]	51	-------	25	18	10	7	6	0	41	1	0	2	1	1	5	71	64.8
Victor × Markton	53	-------	19	12	10	2	3	2	29	0	0	0	1	0	1	49	61.2
Gothland × Markton	56	-------	23	29	17	7	5	3	61	0	0	0	0	0	0	84	72.6
Monarch × Markton	60	-------	19	7	1	1	0	0	9	0	0	0	0	0	0	28	32.1
U. avenae:																	
Canadian × Markton	50	8.6	5	9	3	1	0	0	13	0	0	0	0	0	0	18	72.2
Early Champion × Markton	51	12.5	30	21	9	5	3	0	38	0	0	0	0	0	0	68	55.9
Victor × Markton	53	12.1	24	11	10	1	0	0	22	0	1	0	0	0	[4] 1	47	48.9
Gothland × Markton	56	17.1	22	16	5	1	1	0	23	1	0	0	0	0	[4] 1	46	52.2
Monarch × Markton	60	1.3	18	1	2	1	0	0	4	0	0	0	0	0	0	22	18.2
U. levis:																	
Canadian × Markton	50	32.5	5	4	3	2	2	1	12	1	0	0	0	0	1	18	72.2
Early Champion × Markton [3]	51	28.3	24	22	6	3	1	0	32	0	1	0	0	0	1	57	57.9
Victor × Markton	53	23.1	19	5	7	2	0	0	14	0	0	0	1	0	1	34	44.1
Gothland × Markton	56	5.6	14	17	9	2	1	0	29	1	0	0	0	0	1	44	68.2
Monarch × Markton	60	22.0	27	1	1	0	0	0	2	0	0	0	0	0	0	29	6.9

			REACTION TO U. LEVIS														
Not inoculated:																	
Canadian × Markton	50		21	17	11	4	3	1	36	1	0	0	0	0	1	58	63.8
Early Champion × Markton [3]	51		16	21	11	5	9	3	49	3	3	0	1	0	7	72	77.8
Victor × Markton	53		19	13	5	7	4	2	31	1	0	0	0	0	1	51	62.7
Gothland × Markton	56		22	6	1	1	0	0	8	0	0	0	0	0	0	30	26.7
Monarch × Markton	60		31	33	19	11	7	10	80	6	4	0	0	0	10	121	74.4
U. avenae:																	
Canadian × Markton	56	8.6	9	3	4	0	2	0	9	0	0	0	0	0	0	18	50.0
Early Champion × Markton [3]	51	12.5	28	26	5	5	3	0	39	1	0	0	0	0	1	68	58.8
Victor × Markton	53	12.1	25	11	6	1	2	1	21	0	1	0	0	0	1	47	46.8
Gothland × Markton	56	17.1	35	8	1	0	1	0	10	1	0	0	0	0	1	46	23.9
Monarch × Markton	60	1.3	6	5	1	4	3	2	15	0	0	1	0	0	1	22	72.7
U. levis:																	
Canadian × Markton	50	32.5	6	6	2	2	1	0	11	1	0	0	0	0	[4] 1	18	66.7
Early Champion × Markton [3]	51	28.3	27	17	6	4	2	1	30	0	0	0	0	0	0	57	52.6
Victor × Markton	53	23.1	20	8	3	2	0	1	14	0	0	0	0	0	0	34	41.2
Gothland × Markton	56	5.6	29	6	5	3	1	0	15	0	0	0	0	0	0	44	34.1
Monarch × Markton	60	22.0	9	9	6	4	0	1	20	0	0	0	0	0	0	29	69.0

[1] Smut infection obtained in F_2 as indicated in table 1.
[2] 0=resistant; 1–50=segregating; 51–100=susceptible.
[3] Including data for the reciprocal (hybrid 52).
[4] May represent an escape in F_2.

Before F_2 results are compared with those obtained for F_3 it should be stated that, insofar as it has been possible to determine, no marked difference in the behavior of the F_3 progenies of hybrids 51 (Markton × Early Champion) and 52 (Early Champion × Markton) has been observed. Accordingly, the results from the two hybrids have been combined for study.

In general, the chief genetic interest is found in the data for the F_2 generation and for the first group of F_3 progenies, that is, those descended from uninoculated F_2 plants. All three types of progenies, resistant, segregating, and susceptible, may be expected, since no susceptible F_2 plants have been eliminated. The uninoculated group is mainly considered in the paragraphs that follow; brief references also are made to the reactions of the other two groups of progenies, namely, those descended from F_2 plants inoculated with loose smut and covered smut.

INTERPRETATION OF THE REACTION OF THE HYBRIDS TO USTILAGO AVENAE

Hybrid 50, Canadian × Markton.—Of the 81 F_2 plants inoculated with *Ustilago avenae*, 8.6 percent were infected. In the F_3, 56.9 percent of the progenies descended from uninoculated F_2 plants contained smutted plants. The data for both the F_2 and F_3 generations suggest a possible two-factor relation. If two independent factors for resistance are concerned, 6.25 percent of the F_2 plants should be infected and 56.5 percent of the F_3 progenies should contain smutted plants, very few of which, however, would be classified as susceptible.

Hybrids 51 and 52, Early Champion × Markton and the reciprocal.—Altogether, 305 F_2 plants of the two hybrids were inoculated, of which 38, or 12.5 percent, were infected. In all, 64.8 percent of the F_3 progenies descended from uninoculated F_2 plants contained smutted individuals. These results may suggest a single-factor interpretation, although the number of infected F_2 plants was only half of the expectation. Further, about 75 percent of the F_3 progenies would be expected to contain smutted plants, and approximately one-third of these should be highly susceptible.

Hybrid 53, Victor × Markton.—In the F_2 generation of this cross 12.1 percent of the plants were infected and 61.2 percent of the F_3 progenies descended from uninoculated F_2 plants contained smutted individuals. These results likewise indicate a monogenic type of inheritance for loose smut.

Hybrid 56, Gothland × Markton.—Of this cross 17.1 percent of the F_2 plants were infected. No susceptible F_3 progenies descended from uninoculated, F_2 plants were recorded, although 72.6 percent of these progenies contained smutted individuals. The results with both the F_2 and F_3 generations may suggest a single-factor difference, but the complete lack of susceptible F_3 progenies does not conform with this interpretation.

Hybrid 60, Monarch × Markton.—Since both parental varieties are resistant to the loose smut, it is of special interest that 2 F_2 plants out of a total of 158 grown (1.3 percent) were infected. Further, out of the 28 F_3 progenies grown from uninoculated F_2 plants, 9 contained a few infected individuals, the remaining 19 being fully

resistant. The results for both generations may suggest a three-factor difference.

The reaction to *Ustilago avenae* of the other two groups of F_3 progenies descended from F_2 plants inoculated with *U. avenae* and *U. levis*, respectively, may be briefly considered. Since the second group of F_3 progenies was descended from F_2 plants inoculated with loose smut, fully susceptible progenies would be absent unless a susceptible F_2 plant escaped infection. The reaction of the F_3 progenies of this group is summarized in table 2.

Lack of space forbids the presentation of the data in sufficient detail to bring out possible correlations in the behavior of the different hybrids to both smuts, yet there is no evidence of a similarity in the reaction of the F_3 progenies to the two pathogens. All the data obtained indicate a complete independence in their behavior to either smut. Thus, an F_2 plant might be resistant to one of the smuts and resistant, heterozygous, or susceptible to the other. These facts must be kept fully in mind when considering the data obtained with the loose smut for the F_3 progenies (third group) descended from F_2 plants inoculated with covered smut. A summary of the behavior of these progenies is given in table 2.

INTERPRETATION OF THE REACTION OF THE HYBRIDS TO USTILAGO LEVIS

Hybrid 50, Canadian × *Markton.*—Of the 80 F_2 plants inoculated with *Ustilago levis*, 32.5 percent were infected. Twenty-one F_3 progenies descended from uninoculated F_2 plants were classed as resistant, 36 as segregating, and 1 as susceptible, the latter giving 54.1 percent infection. The data for the two generations do not agree very well. Results with the F_2 plants might suggest a single-factor difference. In this case, however, many more F_3 progenies would be expected to contain infected plants, and approximately one-fourth of them should be highly susceptible.

Hybrids 51 and 52, Early Champion × *Markton.*—There were grown 247 F_2 plants, of which 28.3 percent became infected when inoculated with *Ustilago levis*. Of 72 F_3 progenies descended from uninoculated F_2 plants 16, were classed as resistant, 49 as segregating, and 7 as susceptible, the highest percentage of infection obtained being 88.2 percent. In these hybrids also the F_2 data may indicate a single-factor difference. The data for the F_3 generation likewise are in harmony with this interpretation, except that too few highly susceptible progenies were obtained.

Hybrid 53, Victor × *Markton.*—In the F_2 generation 23.1 percent of the plants became infected when inoculated with *Ustilago levis*. Of 51 F_3 progenies, descended from uninoculated F_2 plants, 19 were classed as resistant, 31 as segregating, and 1 as susceptible, the latter giving 58.3 percent infection. Results with this hybrid correspond rather closely with those obtained for hybrid 50. The F_2 data might indicate a single-factor difference. The data for the F_3 generation, however, are not altogether in agreement with this interpretation, as there are too many resistant progenies and a decided lack of highly susceptible ones.

Hybrid 56, Gothland × *Markton.*—Since Gothland and Markton are both extremely resistant to covered smut, no infection among the F_2

plants would be expected. Actually, however, 9 out of 161, or 5.6 percent, were smutted. In the F_3 generation descended from uninoculated F_2 plants, 30 progenies were grown, of which 22 were classed as resistant and 8 as segregating, all giving a low percentage of infection. The results for this hybrid with covered smut are similar to those obtained for hybrid 60 with loose smut.

Hybrid 60, Monarch × Markton.—There were 246 F_2 plants inoculated, of which 54, or 22 percent, were infected. Of 121 F_3 progenies grown from uninoculated F_2 plants, 31 were classed as resistant, 80 as segregating, and 10 as susceptible, infection in the latter ranging from 50.1 to 66.6 percent. The data for both F_2 and F_3 generations may suggest a one-factor difference in this particular cross. Again, however, there is a marked shortage of susceptible F_3 progenies.

F_3 progenies were grown from F_2 plants belonging to each of the other two groups, inoculated with *Ustilago avenae* and *U. levis*, respectively. The reactions to *U. levis* of these F_3 progenies are summarized in table 2.

In both smut series there was a marked shortage of susceptible progenies and in all hybrids there was a strong preponderance of resistant ones.

Hybrid 56 (Gothland × Markton) and hybrid 60 (Monarch × Markton) afford a number of interesting contrasts. In hybrid 56 one of the parents is resistant to covered smut and susceptible to loose smut, while in hybrid 60 the opposite relation obtains.

The reaction of hybrid 56 to loose smut fits fairly well a simple factorial relation, as 17 percent of the F_2 plants were infected when inoculated with *Ustilago avenae*, and approximately 75 percent of the F_3 progenies contained smutted individuals that were descended from uninoculated F_2 plants. Among the latter, however, fully susceptible progenies were entirely lacking. In the covered smut series, 5.6 percent of the F_2 plants were infected and a small number of F_3 progenies contained smutted individuals. Thus, although the two parents were highly resistant, some infected individuals occurred in both the F_2 and F_3 generations. This may be explained on the basis of three factors, one introduced by one parent and two by the other.

Hybrid 60 has shown a reverse reaction. In the covered smut series, 22 percent of the F_2 plants were infected, and resistant, segregating, and susceptible progenies descended from uninoculated F_2 plants were observed in the F_3 generation. The results suggested a single-factor relation, although there was a shortage of susceptible progenies. In the loose smut series only 1.3 percent of the F_2 plants were infected, and a small number of F_3 progenies descended from uninoculated F_2 plants contained a few smutted individuals.

REACTION OF THE F_4 GENERATION

A large number of F_4 families have been grown in the course of the experiments. These were descended from F_3 progenies of which the reaction to both loose and covered smuts was known. Most of the F_4 families were descended from resistant F_3 progenies. It is impossible to consider here all the data obtained, but some of the more significant results are summarized in table 3.

TABLE 3.—*Summary of data on F_4 progenies of hybrids between Markton and five varieties of oats*

[All F_4 families are descended from F_3 progenies resistant to both *Ustilago avenae* and *U. levis*, except as noted]

Hybrid No.	F_3 progenies	F_4 progenies having indicated reaction to—					
		Ustilago avenae			*Ustilago levis*		
		Resistant	Infected	Range of infection	Resistant	Infected	Range of infection
	Number	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
DESCENDED FROM UNINOCULATED F_2 PLANTS							
50	4	21	0	----------	21	0	----------
51	8	52	9	5.2–29.4	57	4	5.0–35.0
53	7	46	0	----------	45	1	16.6
56	[1] 8	46	4	5.0–25.0	[2] 18	2	5.5– 5.8
60	[1] 4	27	0	----------	[3] 61	3	5.5– 6.6
DESCENDED FROM F_2 PLANTS INOCULATED WITH U. AVENAE							
50	5	13	2	8.3–100	14	1	46.6
51	16	35	13	5.2–53.3	33	15	4.1–16.6
53	14	35	7	5.2–43.7	34	8	4.7–28.0
DESCENDED FROM F_2 PLANTS INOCULATED WITH U. LEVIS							
50	4	10	2	6.2– 6.6	12	0	----------
51	14	31	11	4.3–28.5	38	4	5.0–33.3
53	11	26	6	4.7– 7.6	25	7	4.1–10.3

[1] Resistant to *U. avenae*.
[2] Descended from 4 F_3 progenies resistant to *U. levis*.
[3] Descended from 16 F_3 progenies resistant to *U. levis*.

Most of the F_4 families were completely resistant. In several cases, however, a single plant was infected. A few families gave a somewhat higher percentage of infection. In the F_4 only two families of the total number grown were classified as susceptible, one of these giving 53.3 percent and the other 100 percent infection. These were both in the loose smut series. It is quite possible that in some cases a retest of the F_3 progeny would show that it was actually segregating instead of resistant.

DISCUSSION

Hybrids 50, 51, 52, and 53 involve crosses in which one parent is susceptible to both loose and covered smuts, while the other parent, Markton, is resistant to both. As noted above, all the data obtained indicate a complete independence in the reaction of these hybirds to loose and covered smuts. Accordingly, a progeny resistant to one smut may be resistant, segregating, or susceptible to the other smut.

In all these hybirds there were low percentages (8.6 to 16.0) of F_2 plants infected with the loose smut. On the other hand, relatively high percentages of infection (23.1 in hybrid 53 to 32.5 in hybrid 50) were obtained with covered smut.

In both series of F_3 progenies there were very few susceptible to either loose or covered smut. A failure to obtain susceptible progenies

in the groups descended from uninoculated F_2 plants probably is not due in any way to the methods employed, since, in the parallel series, the susceptible parental varieties regularly gave very high percentages of infection.

The data do not fit well any definite factorial ratio for the inheritance of smut resistance. It may be that the inheritance of resistance to loose smut depends primarily on two main factors. On the other hand, a single-factor difference may account for the results obtained on the inheritance of covered smut.

Several investigators have studied inheritance of resistance in hybrids in which the Markton oat has been used as one parent. Stanton, Reed, and Coffman (*11*) have reported results with Markton × Black Mesdag, Markton × Iogold, Cornellian × Markton, and Richland × Markton. The F_2 and F_3 generations were inoculated with the same races of loose and covered smuts used in the present investigations, and the same methods were employed. Markton and Black Mesdag proved to be very resistant to loose smut in all experiments. In hybrid populations resulting from crossing these varieties, however, one infected F_2 plant was observed, and in the F_3 generation a few progenies contained a small number of infected plants. Iogold and Richland as parents produced rather low percentages of infection with the loose smut, and, when crossed on Markton, a few infected F_2 plants were observed. Several F_3 progenies with smutted individuals also were recorded. Cornellian is moderately susceptible to loose smut, and in the F_2 generation of hybrids on Markton, 9 percent of the plants were infected. Approximately 50 percent of the F_3 progenies proved to be resistant, while the others contained infected individuals, a few being classified as susceptible.

Schattenberg (*9*) studied hybrids between Markton and three other varieties. He used two distinct strains of loose smut, one of which produced a comparatively weak infection of the susceptible parental varieties and the other a more severe infection. He explains his data for the F_3 progenies inoculated with the less virulent strain of smut on the basis of a three-factor difference, and the results obtained agree fairly well with expectations. Nevertheless, there was a shortage of susceptible progenies, with a corresponding slight excess of resistant ones. No clearly defined behavior was obtained with the other strain of smut.

The resistance to covered smut in hybrids in which Markton is one of the parents has been studied by Coffman et al. (*2*). The F_3 progenies of hybrids between Early Champion, Ligowa, Swedish Select, and Markton showed approximately one free from smut to three that contained infected individuals. Very few of these, however, contained highly susceptible families. The data for hybrids 50, 51, 52, and 53, as recorded herein, correspond rather closely with these results, whereas, in the hybrids of Scottish Chief, Iogold, Aurora, and Victory on Markton, about half the progenies were resistant while the other half contained infected individuals. The first three parental varieties of this second group showed comparatively low percentages of infection; Victory, however, proved very susceptible. Finally, in hybrids of Markton with Idamine and Silvermine, a larger proportion of resistant progenies was observed. In comparative experiments higher percentages of infection on an average were obtained from

Idamine than from Silvermine. These varieties are closely related and should have shown similar behavior.

Stanton, Reed, and Coffman (*11*) also recorded data on the inheritance of covered smut for certain hybrids involving Markton. No infected F_2 plants were observed in the Markton × Black Medsag hybrids, but in the F_3 generation infected individuals appeared in a few progenies. The crosses involving Iogold and Richland contained a few infected F_2 plants and also F_3 progenies with smutted individuals. An occasional F_2 plant of Cornellian × Markton was infected, and a few F_3 progenies contained smutted plants.

All investigators of crosses involving Markton have found a great preponderance of resistant F_3 progenies and a corresponding lack of susceptible ones. In a very few cases the results might be accounted for on the basis of a single-factor difference; in others at least two or three independent factors are involved.

The fact is mentioned earlier in this paper that in Markton, and especially in many of the F_3 plants from crosses on Markton, a blasted condition was observed, and apparently much of this blasting was due to the presence of the smut organism in the tissue of the plant. In view of this observed reaction, there perhaps might have been expected a much greater proportion of fully infected plants in the various hybrid generations. Evidently there is some factor or factors that prevent the full expression of susceptibility in hybrids involving Markton.

Results obtained from these hybrids on Markton afford an interesting contrast with those that have been recorded in crosses on Black Mesdag, a variety very resistant to the races of loose and covered smuts used in the present experiments. Reed (*7*) has recorded data for hybrids of Black Mesdag with a hull-less oat and with the Early Champion and Silvermine varieties, all three of which are very susceptible to both smuts. The data for the F_2 generations of the three hybrids indicated that resistance was dominant and that segregation for both smuts occurred on the basis of a 3 to 1 ratio. Furthermore, the data for the F_3 progenies harmonize quite well with those obtained for the F_2 generation. Again, there was a marked parallelism in inheritance of resistance to both smuts in the various F_3 progenies; a progeny resistant, segregating, or susceptible to one smut reacted similarly to the other smut. These results indicate that the same factor or closely linked factors were responsible for the resistance and susceptibility in these hybrids.

Stanton, Reed, and Coffman (*11*) reported results with hybrids between Black Mesdag and Monarch Selection (of Etheridge), the latter susceptible to the loose smut but resistant to the covered. The F_2 and F_3 data indicated segregation on the basis of a 3 to 1 ratio.

Reed (*8*) has studied crosses between Fulghum and Black Mesdag with reference to their reaction to the Fulghum race of loose smut. The results obtained indicated that resistance is dominant and that segregation occurred on the basis of a 3 to 1 ratio, the data for the F_2 and F_3 generations being in harmony.

Austin and Robertson (*1*) have recently reported a two-factor difference in the inheritance of resistance to covered smut in progenies of a Colorado No. 37 × Markton cross. Their F_2 data, however, indicate a three-factor relation. The data presented for the F_3

progenies also fit this interpretation, since 269 were fully resistant and 203 contained infected plants; therefore, a ratio of approximately 37 resistant to 27 segregating and susceptible is demonstrated. Since the publication of their results, Austin and Robertson [4] also have recognized the correctness of the three-factor interpretation.

SUMMARY

This paper presents the results obtained from five oat hybrid combinations involving crosses between Markton and varieties that differ in their behavior to the Missouri races of the loose and covered smuts. Markton is very resistant to both smuts and the crosses are arranged in three groups, depending upon the reaction of the other parential varieties to the smuts: (1) Varieties susceptible to both smuts, (2) a variety susceptible to the loose smut and resistant to the covered smut, and (3) a variety resistant to the loose smut and susceptible to the covered smut.

In group 1 there were four crosses, namely, Canadian × Markton, Early Champion × Markton and its reciprocal, and Victor × Markton. Separate series of F_2 plants of these crosses were inoculated with *Ustilago avenae*-Missouri and *U. levis*-Missouri, respectively. In the loose smut series the percentages of infection of the F_2 plants ranged from 8.6 to 16.0. Higher percentages of infection were obtained in the series inoculated with covered smut, these ranging from 20 to 32.5. Numerous F_3 progenies of each hybrid were inoculated with both loose and covered smuts. The results showed no agreement in the reaction of the progenies to the smuts; they reacted in a manner entirely independent of each other. The data for both F_2 and F_3 generations with loose smut may suggest a two-factor difference, while the corresponding data for the covered smut may suggest a single-factor difference. In both series, however, there was a noticeable lack of fully susceptible progenies.

Hybrid 56, Gothland × Markton, represents the second type of cross. The F_2 plants inoculated with loose smut gave 17.1 percent infection; in the F_3 generation, 72.6 percent of the F_3 progenies descended from uninoculated F_2 plants were segregating, the others resistant. No completely susceptible progenies were observed. Although both parents were resistant to covered smut, there were a few infected F_2 plants and some of the F_3 progenies contained smutted individuals.

Hybrid 60, Monarch × Markton, represents the third group. Although the parents were resistant to loose smut, a few F_2 plants were infected and smutted plants were found also among several F_3 progenies. In the case of the covered smut, 22 percent of the F_2 plants were infected and 74.4 percent of the F_3 progenies descended from uninoculated F_2 plants contained smutted individuals. These data may suggest a single-factor difference, but there is a noticeable lack of susceptible F_3 progenies.

[4] Letter from D. W. Robertson to T. R. Stanton, dated January 15, 1937.

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CHROMOSOMES OF MAIZE FROM NORTH AMERICAN INDIANS[1]

By A. E. LONGLEY

Associate botanist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

A knowledge of the morphological structure of the chromosomes of maize (*Zea mays* L.) and the closely related wild grasses should contribute to an understanding of the origin and distribution of this American cereal.

It was thought that a canvass of the strains of maize grown by the American Indians in isolated localities might disclose types of chromosomes intermediate between those of improved varieties and the wild ancestors of maize. Any additions to the known forms of maize chromosomes would be useful also in cytogenetic studies where there is now a need for visual markers to aid in the identification of parts of chromosomes as they pass from parent to offspring.

McClintock (*4*, *5*, *6*, *7*, *8*, *9*) [2] and others have described the morphological characters by which each chromosome of the set can be identified. The studies of these authors have been based largely on commercial maize or on derived genetic strains. These strains have been separated by many generations from the maize grown by the Indians, and it is possible, therefore, that the studies based on them do not give a complete picture of the morphological characters of maize chromosomes.

With the idea that the maize grown at the present time by the American Indians might have morphological features as yet undescribed, the author undertook to examine the chromosomes of such Indian strains as still are available. It is hoped, as opportunity offers, to extend this study, here limited to the Indian strains of the United States, to the maize of the Indians in Mexico, Central America, and South America.

MATERIAL AND METHODS

The source of material for this paper is a collection of maize from 33 North American Indian tribes of the United States. It should be understood that these tribes have to some extent intermingled as the result of their removal from their original lands to reservations.

Every effort has been made to obtain seed grown by Indians and free from admixture with commercial sorts, but, of course, in many instances there can be no certainty that such admixture has not taken place.

Carmine smears were used by the writer in making pollen mother cell preparations for study. It has been found that the best stained slides come either from fresh material or from material that has been

[1] Received for publication July 21, 1937; issued March 1938

[2] Reference is made by number (italic) to Literature Cited, p. 195.

killed in acetic-absolute alcohol (1:3) and held in absolute alcohol for about a day. Even under optimum conditions the chromosomes of some strains of maize stain with carmine less readily than do those of others. This difference between strains in the staining quality of chromosomes is more apparent in material stored for some time in absolute alcohol.

Identification of the individual chromosomes of the plants of the various strains of maize included in this study is based on their morphological characteristics, a method that may not be infallible. However, to test the chromosomes of each plant or even of each strain by crossing with plants in which each chromosome has a well-known marker would have been such a long process that the writer is presenting the following data without this additional check.

CHROMOSOME MORPHOLOGY

The detailed study of the individual maize chromosomes began with McClintock's attempt (*4*) to identify each chromosome from division phases of the microspore. Later McClintock (*6*) found that the early prophase of the first reduction division of the pollen mother cell was even more favorable for morphological studies. In this and later studies she has shown that every chromosome of the set is morphologically identifiable. These chromosomes have been given numbers from I to X beginning with the longest and ending with the shortest, and by combining cytological and genetic investigations, it has been possible to assign each linkage group in maize to its proper chromosome.

Maize chromosomes as seen in the midprophase of the first meiotic division of the pollen mother cells are in the paired thread condition. Stained with acetocarmine these 10 double threads are seen to differ in length, and each is divided into two arms by a fiber attachment region. In one chromosome these two arms are almost equal in length, but in others they are quite unequal. Knobs, when present at certain positions on these threads, are very useful in identifying the individual chromosomes. These major morphological characters of the maize chromosomes are often associated with other minor features that help to identify more readily and more definitely certain chromosomes.

The following presentation records the number and the position of knobs that aid materially in identifying each of the 10 chromosomes of maize or even fragments of them.

The data from 400 or more plants studied during the past three seasons are much too extensive to be given in detail, so they have been condensed and presented in table 1. In this table are listed the Indian tribes from which maize was obtained and their general location. Each of the 10 chromosomes is assigned a general section, and each section is subdivided into columns designating types 1, 2, 3, and 4. For each chromosome the number of plants without knobs is given in column 1, the number of plants with a knob on the short arm is given in column 2, the number of plants with a knob or knobs on the long arm is given in column 3, and the number of plants with a knob on the short arm and one or more knobs on the long arm is

given in column 4. Column 3 under the heading "Chromosome VIII" and column 4 under the heading "Chromosome VI" are further subdivided, since in the case of each of these chromosomes the long arm may be marked by more than one knob. The subdivision designated "a" gives the number of plants with the chromosome marked by a knob in the position nearest the end; "b", the number of plants with the chromosome marked by a knob in the second position from the end; and "c", the number of plants having the chromosome marked by a knob in the third position from the end. When knobs occur in more than one position on an arm, the number of plants is given under the combinations of ab, ac, bc, or abc, in accordance with the positions occupied.

The percentages at the foot of each of the columns under each chromosome of table 1 show the frequency of the chromosome types

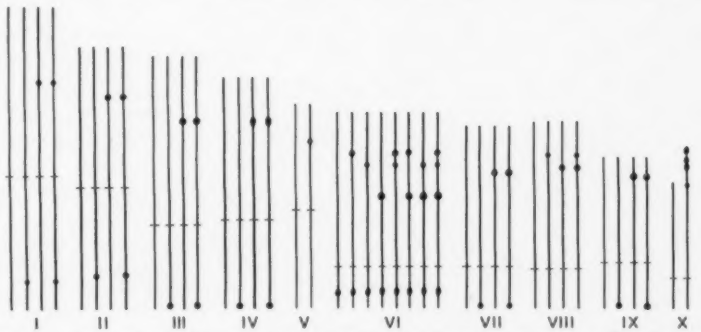

FIGURE 1.—Diagrammatic representation of the 10 chromosomes of maize showing types with or without knobs. Cross line indicates position of attachment of the spindle fiber.

described above, in the plants included in this study. These percentages may have little significance, however, since the number of plants of a strain was often very small.

The significance of the divisions and subdivisions under each chromosome of table 1 is made clearer when these divisions are examined in conjunction with figure 1. This figure presents diagrammatically the variations found in the position of the knobs of each of the 10 chromosomes. It shows also the relative length of each chromosome and the position of the attachment of the spindle fiber.

The size of any given knob on a chromosome, although constant for a particular strain, may vary when this knob is compared with that of an unrelated strain. Figure 1 shows all knobs about the same size, but actually the size may vary greatly.

Neither the data of table 1 nor the diagram of the chromosomes (fig. 1) differentiates between cases in which a knob is present on only one of the paired chromosomes and those in which both members of the pair are marked. This heterozygous condition is frequently difficult to determine and consequently in this general survey such cases are treated as though both chromosomes were marked by knobs at this position.

TABLE 1.—*Number of plants of the various chromosome types in maize strains grown by Indian tribes of different localities*

Indian tribe from which maize was obtained	Locality	Number of plants of indicated type [1] of chromosome—																																						B-type chromosome	
		I				II				III				IV				V		VI							VII				VIII				IX				X			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	3	2	4b	4b	4c	4ab	4ac	4bc	4abc	1	2	3	4	1	3a	3b	3ab	1	2	3	4	1	Abnormal	Without knobs	With knobs
Assiniboine	Montana	4	0	0	0	4	0	0	0	4	0	0	0	4	0	0	0	4	0	4	0	0	0	0	0	0	0	4	0	0	0	4	0	0	0	1	3	0	0	4	0	4	0
Crow	do	4	0	0	0	3	1	0	0	4	0	0	0	4	0	0	0	4	0	4	0	0	0	0	0	0	0	4	0	0	0	4	0	0	0	0	4	0	0	4	0	4	0
Sioux or Cheyenne	South Dakota	10	0	0	0	6	2	0	0	10	0	0	0	8	0	0	0	9	0	6	3	0	0	0	0	0	0	10	0	0	0	9	0	1	0	0	2	9	0	0	0	11	0	7	0
Mandan	North Dakota	11	0	0	0	11	0	0	0	11	0	0	0	11	0	0	0	6	5	3	8	0	0	0	0	0	0	11	0	0	0	11	0	0	0	0	3	8	0	0	0	11	0	11	0
Shoshone	Idaho	10	0	0	0	9	0	0	0	9	0	0	0	9	0	0	0	4	6	6	1	2	0	0	0	0	0	9	2	0	0	10	0	0	0	0	11	0	0	0	0	10	0	11	0
Menominee	Wisconsin	2	0	0	0	2	0	0	0	2	0	0	0	2	0	0	0	0	2	2	0	0	0	0	0	0	0	2	0	0	0	2	0	0	0	0	2	0	0	0	0	2	0	2	0
Sisseton	South Dakota	5	0	0	0	5	0	0	0	5	0	0	0	4	0	0	0	3	0	2	2	0	0	0	0	0	0	5	0	0	0	2	0	0	0	0	5	0	0	0	0	5	0	0	4
Sioux	North and South Dakota.	22	0	0	0	18	4	0	0	22	0	0	0	19	0	3	0	22	0	5	18	0	0	0	0	0	0	21	0	2	0	23	0	0	0	1	21	0	0	23	0	15	7
Ponca	Oklahoma	12	0	0	0	8	0	3	0	11	0	0	0	11	0	0	0	11	0	6	0	0	0	5	0	0	0	10	0	2	0	10	0	0	0	0	11	0	1	12	0	12	0
Potawatomi	Kansas	9	0	0	0	5	0	4	0	9	0	0	0	9	0	0	0	9	0	1	6	2	0	0	0	0	0	8	0	1	0	9	0	0	0	1	8	0	0	9	0	7	2
Sioux or Blackfeet	North Dakota	18	0	0	0	18	0	0	0	17	0	1	0	18	0	0	0	5	11	3	12	2	1	1	0	0	0	16	0	4	0	19	0	0	0	4	16	0	0	19	0	20	2
Chippewa	Minnesota and Wisconsin.	10	0	0	0	7	0	3	0	10	0	0	0	10	0	0	0	5	5	3	4	3	0	0	0	0	0	10	0	0	0	10	0	0	0	0	10	0	0	10	0	9	2
Cheyenne	Montana	2	0	0	0	2	0	0	0	2	0	0	0	0	2	0	0	2	0	1	1	0	0	0	0	0	0	2	0	0	0	2	0	0	0	0	2	0	0	2	0	2	0
Winnebago	Nebraska	12	0	0	0	2	8	2	0	12	0	0	0	12	0	0	0	12	0	4	8	0	0	0	0	0	0	12	0	0	0	11	0	0	0	0	13	0	0	12	0	4	9
Shawnee	Oklahoma	5	0	0	0	4	1	0	0	5	0	0	0	5	0	0	0	1	4	3	0	0	0	2	0	0	0	5	0	0	0	4	0	1	0	0	4	0	0	5	0	5	0
Sac and Fox	Iowa	14	0	0	0	8	3	4	0	14	0	0	0	15	0	0	0	7	7	5	4	0	0	3	0	0	2	10	0	5	0	14	0	0	0	2	11	0	2	15	0	10	5
Cheyenne or Arapahoe	Oklahoma	11	2	0	0	6	0	8	0	13	0	0	0	5	0	8	0	13	0	5	9	0	0	0	0	0	0	7	0	7	0	6	0	7	0	0	5	8	0	0	0	12	3
Seminole	do	4	0	0	0	1	4	0	0	4	0	0	0	0	0	5	0	4	0	0	3	1	0	0	0	0	0	4	0	0	0	4	0	0	0	0	5	0	0	3	0	5	0
Five Civilized Tribes	do	11	0	0	0	9	0	3	0	10	0	0	0	5	0	6	0	4	5	1	5	3	0	2	0	0	0	4	0	0	0	8	0	2	0	0	4	6	0	0	0	10	1
Osage	do	27	0	0	0	18	0	5	1	24	0	2	0	17	7	2	0	16	11	7	3	10	0	3	2	2	0	14	0	12	0	20	0	5	0	1	19	0	0	31	0	32	0
Cherokee	North Carolina	2	0	0	0	0	0	3	0	3	0	0	0	0	0	3	0	0	3	3	0	0	0	0	0	0	0	0	0	2	0	3	0	0	0	0	3	0	0	3	0	5	0
Pawnee	Oklahoma	8	0	2	0	2	4	2	2	10	0	0	2	8	0	2	0	8	3	6	4	0	0	0	0	0	0	7	0	3	0	8	0	2	0	0	9	0	0	11	0	11	0
Mission	California	2	2	0	0	0	0	4	0	2	0	0	0	2	0	0	0	3	0	0	2	2	0	0	0	0	0	2	0	0	0	1	0	2	0	0	4	3	0	3	0	5	0
Kiowa	Oklahoma	2	0	1	0	2	1	0	0	3	0	0	0	2	0	1	0	2	1	0	2	1	0	0	0	0	0	3	0	0	0	2	0	0	0	0	3	0	0	3	0	3	0
Zuni and Tewa	New Mexico	5	0	0	1	5	0	0	1	5	0	1	0	5	0	1	0	5	1	2	1	0	0	3	0	0	0	0	0	7	0	5	1	0	0	1	5	0	0	6	0	6	2
Cherokee	Oklahoma	5	2	0	0	1	3	0	4	6	0	0	0	3	0	4	0	5	2	3	4	0	0	0	0	0	0	3	0	0	0	1	0	0	0	8	1	0	0	9	0	9	0
Walapai	Arizona	12	0	0	0	1	0	10	0	5	0	7	0	5	0	7	0	5	3	5	6	0	0	0	0	0	0	4	0	8	0	7	3	0	0	4	5	0	0	13	0	14	0
Ute	Utah	3	3	0	0	5	0	2	0	2	1	3	3	5	0	4	0	2	6	1	6	0	1	2	2	0	0	6	0	2	0	3	0	0	0	5	6	0	0	3	0	9	0
Mescalero Apache	Arizona	8	3	7	3	4	1	10	8	7	0	8	10	5	1	11	2	5	19	8	7	2	0	2	4	1	0	11	0	12	0	7	0	15	1	6	18	0	0	17	0	21	2
Pima	do	14	2	0	0	0	0	3	7	5	0	11	0	0	0	14	0	0	16	0	1	0	0	16	0	0	0	0	3	7	5	0	0	8	8	0	17	0	0	17	0	17	0

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DESCRIPTION OF CHROMOSOMES

Any description of the chromosomes of maize is naturally built around the descriptions that have been published previously. Although the author appreciates that his colleagues are familiar with many of the different types of each chromosome described in this article, it may be helpful to others to call attention to previously published drawings and diagrams of maize chromosomes.

Chromosome I has been pictured diagrammatically without knobs by both Burnham (*1*) and McClintock (*8*), and by the latter author it has been shown with a small knob terminating the short arm. Mcclintock in three publications (*6*, *7*, *8*) has shown chromosome II with a knob on the long arm. The same author (*6*, *8*) pictures diagram-

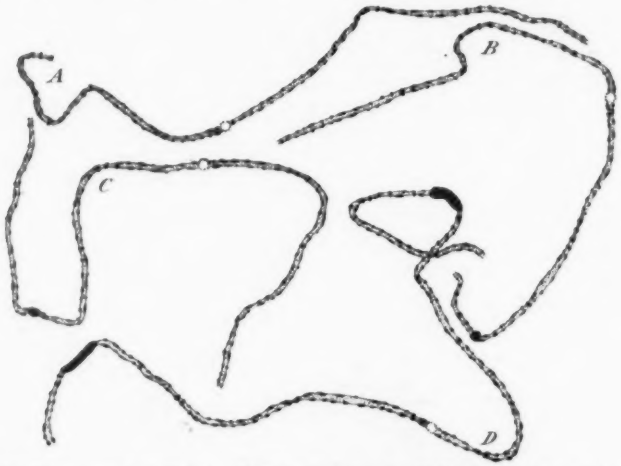

FIGURE 2.—Chromosome I: *A*, Type 1, without knob; *B*, type 2, with knob on short arm; *C*, type 3, with knob on long arm; and *D*, type 4, with knobs on both arms. × 1,500.

matically chromosome III without knobs. Chromosome IV is shown diagrammatically by McClintock (*8*) with a knob on the long arm in its characteristic position. McClintock (*8*) pictures chromosome V with a knob on the long arm and Rhoades (*11*, *12*), in his cytogenetic research, has used both the knobless and the knobbed type of chromosome V.

Burnham (*1*) shows in his diagram of chromosome VI no knobs on its long arm, while McClintock (*6*, *8*, *9*) has pictured this chromosome with both one and two knobs on the long arm. Chromosome VII is shown in McClintock's diagrams (*6*, *8*) with a knob on the long arm and with knobs on both arms. The same author (*5*, *8*) pictures chromosome VIII with one knob and with two knobs, a larger and a smaller one, the latter nearer the end of its long arm. Chromosome IX has types with and without a knob terminating the short arm in the maize studied both by McClintock (*5*, *8*, *9*) and by Creighton and McClintock (*2*). The tenth or shortest chromosome has been shown diagrammatically without knobs by McClintock (*6*, *8*).

CHROMOSOME I

The fact that chromosome I is the longest of the maize chromosomes serves to distinguish it from the other chromosomes. From figures 1 and 2 it is apparent that chromosome I is not always a long chromosome marked only by chromomeres and the spindle fiber attachment but may have in addition a knob or dark staining enlargement near the end of the short arm and another at a position approximately one-fourth the length of the chromosome from the end of the long arm. When this chromosome has a knob or knobs at one or both of these positions, the author has found it rather easy to identify. Frequently this chromosome has the terminal chromomere of the short arm enlarged

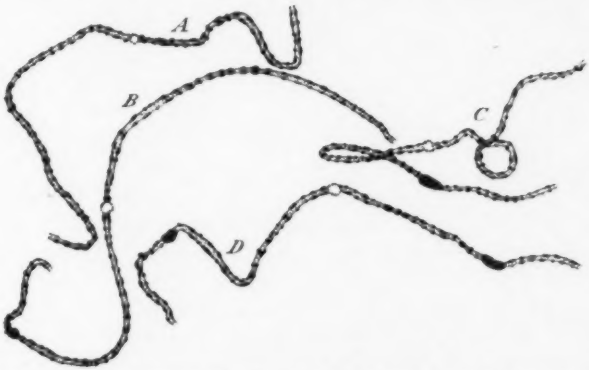

FIGURE 3.—Chromosome II: *A*, Type 1, without knob; *B*, type 2, with knob on short arm; *C*, type 3, with knob on long arm; and *D*, type 4, with knobs on both arms. × 1,500.

sufficiently to be a distinguishing feature, but since the enlargement of this chromomere never seems to be prominent it has not been treated as a knob.

CHROMOSOME II

Four types of chromosome II are shown in figures 1 and 3. This chromosome is most readily identified when it has a knob on both arms. The unmarked type and the type with only a knob on the long arm may be confused with the two types of chromosome V. This confusion disappears when the two chromosomes are carefully scrutinized. Chromosome II has arms more unequal in length, and when the long arm is marked by a knob the knob is a little farther from the end of the chromosome than is the case with chromosome V.

CHROMOSOME III

Of the long chromosomes, chromosome III is most readily identified because the long arm is approximately double the length of the short arm. The four types of this chromosome are shown in figures 1 and 4. When a knob is present on the long arm it is approximately one-fourth of the length of the chromosome from the end. The knob on the short arm has been pictured as terminal, but in those cases in which this knob was reduced in size to a prominent chromomere it was the third chromomere from the end that was enlarged.

CHROMOSOME IV

Chromosome IV has few morphological characteristics to distinguish it sharply from several of the other chromosomes, but in spite of this fact it has never seemed difficult to identify. Figures 1 and 5 show the four types of this chromosome. In those plants in which the short arm showed a knob, this knob was so similar to the knob on the

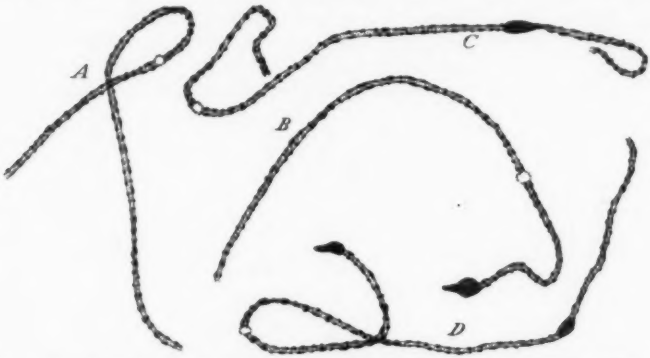

FIGURE 4.—Chromosome III: *A*, Type 1, without knob; *B*, type 2, with knob terminating short arm; *C* type 3, with knob on long arm; and *D*, type 4, with knobs on both arms. × 1,500.

short arm of chromosome III, both in position and in size variations, that for a time the author was somewhat confused, but uncertainty of identification was dispelled when both chromosomes III and IV, with knobs on the short arm, were found in the same plant and were distinguishable not only by the difference in position of the spindle

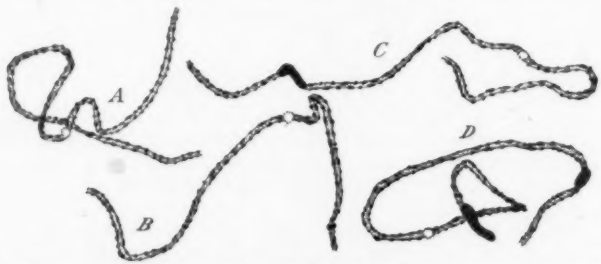

FIGURE 5.—Chromosome IV: *A*, Type 1, without knob; *B*, type 2, with small knob near end of short arm; *C*, type 3, with knob on long arm; and *D*, type 4, with knobs on both arms. × 1,500.

fiber attachment but also by the presence of knobs on the long arms. The knob on this arm of chromosome III is farther from the end than is that on chromosome IV.

CHROMOSOME V

The two arms of chromosome V are nearly equal in length, and it is this characteristic that serves best as an identifying feature. The likelihood of confusing this chromosome with chromosome II has been discussed above. The absence of a knob on the short arm of

chromosome V in any of the Indian strains included in this study leaves only two types of this chromosome to be illustrated in figures 1 and 6.

CHROMOSOME VI

The so-called satellite or sixth chromosome is readily identified because of its attachment at prophase to the nucleolus. Figure 7

FIGURE 6.—Chromosome V: *A*, Type 1, without knob; *B*, type 3, with knob on long arm. × 1,500.

shows the eight types that the author has observed. The long arm of this chromosome is only rarely seen without one or both of the small knobs nearest the distal end, while the knob at about the mid-

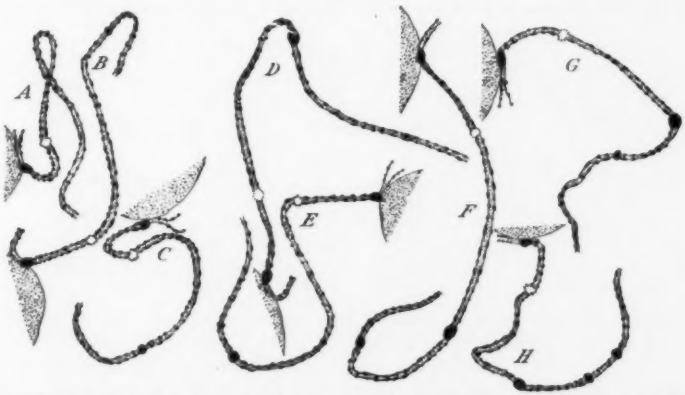

FIGURE 7.—Chromosome VI: *A*, Type 2, without knob on long arm; *B*, type 4a, with knob on long arm in position nearest the end; *C*, type 4b, with knob on long arm in second position from the end; *D*, type 4c, with knob on long arm in third position from the end; *E*, type 4ab, with knobs on long arm in first and second positions; *F*, type 4ac, with knobs on long arm in first and third positions; *G*, type 4bc, with knobs on long arm in second and third positions; and *H*, type 4abc, with three knobs on long arm. × 1,500.

point of this arm, although usually prominent, is less frequently present.

CHROMOSOME VII

McClintock described to the author a universal characteristic of chromosome VII that makes its identification rather simple. On the long arm adjacent to the spindle fiber attachment the chromosome is broad and stains deeply with carmine. Of the four types shown in figures 1 and 8 the types with a knob terminating the short arm are

rarely present and were found in only one of the strains given in table 1.

CHROMOSOME VIII

Chromosome VIII is distinguished most readily from the other chromosomes when it is marked by two knobs on the long arm. These knobs are usually unequal in size and the knob nearer the end is never

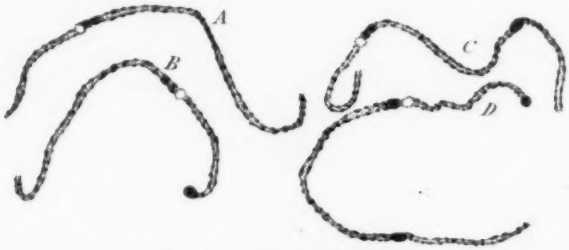

FIGURE 8.—Chromosome VII: *A*, Type 1, without knobs; *B*, type 2, with knob terminating short arm; *C*, type 3, with knob on long arm; and *D*, type 4, with knobs on both arms. × 1,500.

very prominent. Some types of chromosome VIII that are likely to be confused with chromosome VII are distinguishable from them by the absence of the heavily stained region adjacent to the spindle

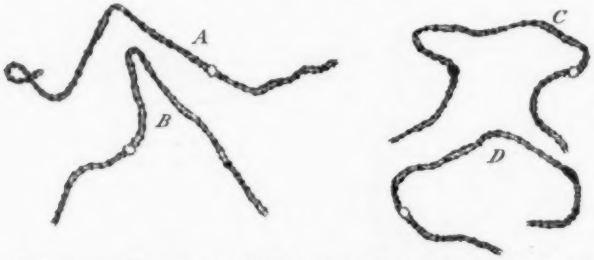

FIGURE 9.—Chromosome VIII: *A*, Type 1, without knob; *B*, type 3a, with small knob at position nearest the end of long arm; *C*, type 3b, with knob at position second from the end of long arm; and *D*, type 3ab, with two knobs on long arm. × 1,500.

fiber attachment. Figure 9 shows the four types that occur in the strains given in table 1.

CHROMOSOME IX

Cytogeneticists have found chromosome IX very useful because it is so readily identified and because its linkage group is one of the best known. This chromosome "stands out" from the shorter just as chromosome III "stands out" from the longer chromosomes because the short arm is approximately one-half the length of the long arm. The knob terminating the short arm, though occurring in several sizes, is so frequently present that it almost leaves the impression that chromosome IX is incomplete without it. Figure 10 shows three types of chromosome IX; the type with a knob near the end of the long arm was found in only a few strains.

CHROMOSOME X

Chromosome X, the shortest of all maize chromosomes, is shown in figure 11, *A*. The author in a few instances has found on this chromosome a prominent chromomere near the end of the long arm, but in the present discussion this enlargement has not been treated as a knob. In figure 11, *B*, is shown an abnormal type of chromosome X, a type similar to that already described by the author (*3*) as occurring in both teosinte and maize. This abnormal type of the tenth chromosome has an additional piece on the end of the long arm, approximately

FIGURE 10.—Chromosome IX: *A*, Type 1, without knob; *F*, type 2, with knob terminating short arm; and *C*, type 4, with knobs on both arms. × 1,500.

the length of the short arm. In some plants this abnormal piece may be marked by a large knob near the end as shown in figure 11, *B;* in other plants this region may show two adjacent knobs instead of the one large knob, and in still other plants there also may be a knob terminating the chromosome (fig. 1). In these abnormal types of chromosome X there are usually one or two knoblike enlargements near the end of the normal part of the chromosome. Additional study of the tenth chromosome may show that these positions on the

FIGURE 11.—Chromosome X: *A*, Normal type; *B*, one of the abnormal types. × 1,500.

normal tenth chromosome have a tendency to form knobs. Nothing in the character of the additional piece found attached to the tenth chromosome has suggested a solution of its origin.

POSITION AND NUMBER OF KNOBS

If in a single plant all positions on the chromosomes that have been observed to have knobs were so occupied, that plant would have 18 knobs. This disregards the knoblike enlargement at the nucleolar attachment region of chromosome VI and considers only the knobless type of chromosome X. Such a plant would have for its chromosome complement the nine right-hand types of the nine longest chromosomes shown in figure 1 and the normal knobless type of the tenth chromosome.

Other knob-forming positions may be found, but the present discussion is limited to the 18 observed positions. In addition to being restricted to definite positions, knobs in some instances are also

characterized by their size and shape, although in other instances the size of a knob differs in different maize strains.

Considering, therefore, these 18 knob positions, it has been found that some plants are without knobs at any of the positions and that other plants may have as many as 14 of the positions marked by knobs. It should be comparatively easy, by means of appropriate combinations, to produce plants with knobs at all 18 positions or to maintain strains without knobs.

To visualize better the relationship between these 18 positions and the data presented in table 1, these data have been tabulated differently in table 2. The values in the 18 columns, under the 9 chromosomes that are marked by knobs, indicate the frequency that each position has been marked by a knob. A frequency of 0 percent indicates that no knob has been found at this position among all the plants of the strain; a frequency of 100 percent indicates that a knob has been found at this position in all plants of the strain.

If a strain of maize were uniform all 18 positions would show a frequency for each position of either 0 or 100 and never an intermediate value. Such uniformity may be reached by prolonged pure seeding or selfing. The author's material was not taken from pure lines and frequently the data for a strain combine the data from several substrains that, although coming from the same Indian tribe, may have been received at different times and from different localities. Consequently similar and not identical morphological markers on the chromosomes were to be expected in any particular maize strain.

TABLE 2.—*Percentage of plants with knobs at each of the 18 positions on 9 of the 10 chromosomes of maize from 33 Indian tribes*

Indian tribe from which maize was obtained	Percentage of plants having specified chromosome with knobs at indicated position								
	I		II		III		IV		V
	Short arm	Long arm	Short arm	Long arm	Short arm	Long arm	Short arm	Long arm	Long arm
Assiniboine	0	0	0	0	0	0	0	0	0
Crow	0	0	25	0	0	0	0	0	0
Sioux or Cheyenne	0	0	25	0	0	0	0	0	0
Mandan	0	0	0	0	0	0	0	0	45
Shoshone	0	0	0	0	0	0	0	0	60
Menominee	0	0	0	0	0	0	0	0	100
Sisseton	0	0	0	0	0	0	0	0	0
Sioux	0	0	18	0	0	0	0	14	0
Ponca	0	0	0	27	0	0	0	0	0
Potawatomi	0	0	0	44	0	0	0	0	0
Sioux or Blackfeet	0	0	0	0	0	6	0	0	69
Chippewa	0	0	0	30	0	0	0	0	50
Cheyenne	0	0	0	0	0	0	100	0	0
Winnebago	0	0	67	17	0	0	0	0	0
Shawnee	0	0	20	0	0	0	0	0	80
Sac and Fox	0	0	20	27	0	0	0	0	50
Cheyenne or Arapahoe	15	0	0	57	0	0	0	62	0
Seminole	0	0	80	0	0	0	0	100	0
Five Civilized Tribes	0	0	0	25	0	0	0	54	56
Osage	0	0	4	25	0	8	27	8	41
Cherokee (North Carolina)	0	0	0	100	0	0	0	100	100
Pawnee	0	20	60	40	17	17	0	20	27
Mission	50	0	0	100	0	0	0	0	0
Kiowa	0	33	33	0	0	0	0	33	33
Zuni and Tewa	17	17	17	17	0	17	0	17	17
Cherokee (Oklahoma)	28	0	88	50	0	0	0	57	28
Walapai	0	0	0	91	0	58	0	50	38
Ute	50	0	0	28	44	67	0	44	75
Mescalero Apache	28	48	39	78	40	72	16	68	79
Pima	12	0	70	100	0	69	0	100	100
Hopi	100	43	80	40	0	100	0	100	100
Pueblo	100	100	100	75	0	100	0	80	100
Navajo	79	54	90	92	26	95	0	98	96

TABLE 2.—*Percentage of plants with knobs at each of the 18 positions on 9 of the 10 chromosomes of maize from 33 Indian tribes*—Continued

Indian tribe from which maize was obtained	Percentage of plants having specified chromosome with knobs at indicated position									Average number of knobs per plant
	VI			VII		VIII		IX		
	Long arm			Short arm	Long arm	Long arm		Short arm	Long arm	
	a [1]	b [2]	c [3]			a [1]	b [2]			
Assiniboine	0	0	0	0	0	0	0	75	0	0.75
Crow	0	0	0	0	0	0	0	100	0	1.25
Sioux or Cheyenne	33	0	0	0	0	0	10	82	0	1.50
Mandan	73	0	0	0	0	0	0	73	0	1.91
Shoshone	11	22	0	0	0	0	0	100	0	1.93
Menominee	0	0	0	0	0	0	0	100	0	2.00
Sisseton	50	0	0	0	0	0	50	100	0	2.00
Sioux	78	0	0	0	10	0	0	96	0	2.16
Ponca	45	45	0	0	17	0	0	100	8	2.42
Potawatomi	67	22	0	0	11	0	0	89	0	2.33
Sioux or Blackfeet	68	16	5	0	20	0	0	80	0	2.64
Chippewa	40	30	0	0	0	0	0	100	0	2.50
Cheyenne	50	0	0	0	0	0	0	100	0	2.50
Winnebago	67	0	0	0	0	0	0	100	0	2.51
Shawnee	40	40	0	0	0	0	20	80	0	2.80
Sac and Fox	64	36	14	0	33	0	0	87	13	3.44
Cheyenne or Arapahoe	64	0	0	0	50	0	54	62	0	3.64
Seminole	75	25	0	0	0	0	0	100	0	3.80
Five Civilized Tribes	64	45	0	0	56	0	20	60	0	3.80
Osage	30	56	15	0	46	0	20	97	31	4.08
Cherokee (North Carolina)	0	0	0	0	100	0	0	0	0	4.00
Pawnee	40	0	0	0	30	0	20	100	25	4.16
Mission	50	50	0	0	0	66	0	100	0	4.16
Kiowa	67	33	0	0	0	0	100	100	0	4.32
Zuni and Tewa	67	50	0	0	100	0	17	83	0	4.36
Cherokee (Oklahoma)	57	0	0	0	62	0	86	11	0	4.67
Walapai	54	0	0	0	67	0	42	64	18	4.82
Ute	80	20	10	0	75	56	67	80	20	7.16
Mescalero Apache	56	24	24	0	52	4	70	75	0	7.73
Pima	100	94	0	53	80	50	100	100	0	10.28
Hopi	86	43	100	0	80	0	100	78	0	10.50
Pueblo	100	33	33	0	100	0	100	100	33	11.54
Navajo	81	74	40	0	98	42	98	91	37	11.91

[1] Knob position nearest the end.
[2] Knob position second from the end.
[3] Knob position third from the end.

The average number of positions marked by knobs in a given strain is shown in the last column of table 2. Several methods were tried to derive a satisfactory average for the number of knobs per chromosome per plant and per strain. If the author had been able to make a drawing of every chromosome of all plants the total number of knobs on the 10 chromosomes of each plant of a strain could have been averaged, but to drop out all plants of a strain that did not have a drawing of every chromosome was unsatisfactory because in so many instances those plants with a drawing of all 10 chromosomes were the plants with the fewer knobs on the chromosomes. Consequently an attempt was made to devise a method that would combine the complete data from some plants with the fragmentary data from others.

A fairer method that would use data from all plants whether complete or fragmentary seemed to be to consider each knob position separately, as in table 2. This method takes into account each knob position on each chromosome, and when a particular knob position on a chromosome is marked by a knob in all plants of a strain the knob frequency is 100 percent for this position, while if this

position is never marked by a knob the frequency is 0. In many instances part of the plants of a strain have a knob at a certain position and part are knobless, thus giving a knob frequency intermediate between 0 and 100 percent. The sum of the knob frequency of all 18 positions thus determined independently is taken as the average frequency of knobs for a strain.

Averaging the number of knobs on the chromosomes of a strain should be restricted, of course, to those strains in which the number of knobs on the chromosomes of the individual plants have a random distribution about their mean. The number of plants studied from a strain was frequently too meager to test such a distribution. In a few strains, however, it was evident that certain substrains should not be combined. The data from such strains that have been averaged in table 2 have also been subdivided and presented in table 3.

TABLE 3.—*Percentage of plants with knobs at each of the 18 positions on 9 of the 10 chromosomes of maize from four Indian strains*

Indian tribe from which maize was obtained	Percentage of plants having specified chromosome with knobs at indicated position								
	I		II		III		IV		V
	Short arm	Long arm	Short arm	Long arm	Short arm	Long arm	Short arm	Long arm	Long arm
Ute	0	0	0	0	0	0	0	0	0
Pawnee	0	0	50	25	0	0	0	0	0
Mescalero Apache	0	0	0	0	0	0	0	0	0
Zuni and Tewa	0	0	0	0	0	0	0	0	0
Mescalero Apache	0	38	12	75	20	50	0	83	70
Ute	75	0	0	40	57	86	0	57	100
Mescalero Apache	54	64	62	92	62	100	27	73	100
Pawnee	0	100	100	100	100	100	0	100	100
Tewa and Zuni	100	100	100	100	0	100	0	100	100

Indian tribe from which maize was obtained	Percentage of plants having specified chromosome with knobs at indicated position									Average number of knobs per plant
	VI			VII		VIII		IX		
	Long arm			Short arm	Long arm	Long arm		Short arm	Long arm	
	a[1]	b[2]	c[3]			a[1]	b[2]			
Ute	100	0	0	0	0	0	0	0	0	1.00
Pawnee	25	0	0	0	0	0	0	100	0	2.00
Mescalero Apache	0	100	0	0	0	0	0	100	0	2.00
Zuni and Tewa	60	40	0	0	100	0	0	80	0	2.80
Mescalero Apache	50	0	0	0	0	0	70	33	0	5.01
Ute	75	25	12	0	33	71	86	100	25	8.42
Mescalero Apache	69	31	46	0	100	9	82	100	0	10.71
Pawnee	100	0	0	0	100	0	100	100	100	12.00
Tewa and Zuni	100	100	0	0	100	0	100	100	0	12.00

[1] a=Knob position nearest the end.
[2] b=Knob position second from the end.
[3] c=Knob position third from the end.

By the foregoing method the author has attempted to arrive at a figure from the data available that may be used as a measure of the knobbiness of the chromosomes in each of the 33 strains of Indian corn. The values in the last column of table 2 showing the average

number of knobs for each of the 33 strains are also presented graphically in figure 12. The range is from less than 1 to almost 12 knobs.

Maize from the Pima, Hopi, Pueblo, and Navajo Indians is shown in table 2 as having an average number of knobs per plant greater than 10. This group coming from adjoining regions suggests a source of material should prominent knob markers on most of the chromosomes be desired.

The same tendency toward knobs is found in most of the plants from the Mescalero Apache and in some plants from the Zuni and

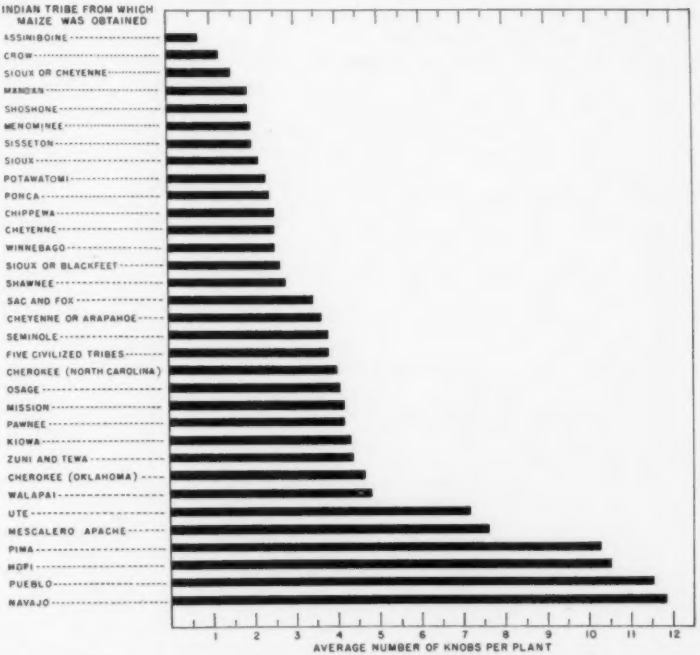

FIGURE 12.—Average number of knobs per plant on the chromosomes of 33 strains of Indian corn.

Tewa,[3] Ute, and Pawnee Indians. These strains are the four that have been subdivided in table 3 because the plants with many knobs were clearly separated from those with few knobs.

In marked contrast to the prevalence of knobs on the chromosomes of maize from these southwestern localities is the small number of knobs on the maize from the Indians along the northern border of the United States. Of the first 16 tribes listed in table 2, 14 are northern tribes. All of these except the Shoshone belong either to the Siouan or to the Algonquin families. The 14 strains of maize from these northern tribes have very few knobs on the chromosomes, and the whole range is only from 0.75 to 3.44 knobs per plant.

Nine strains of maize given in table 1 are from Indians of Oklahoma, the melting pot of the northern and eastern Indian tribes. Table 2

[3] Seed from this strain was collected from several reservations and then grown in Colorado.

shows that the number of knobs on the chromosomes of these nine Oklahoma strains ranges from 2.42 to 4.67 per plant. These strains, although intermediate in geographical position between those of the northern and those of the southwestern Indian tribes described above, are not intermediate when the number of knobs on the chromosomes of the plants is compared.

Only one strain of maize from the Southeast is shown in table 2. The average number of knobs on the chromosomes of maize of the Cherokee Indians from North Carolina is four per plant. It is scarcely justifiable to use the data from this one strain as indicative of the morphological characteristics of the chromosomes of the Indian corn of the entire southeastern area. This one example, when combined with data from the southeastern tribes now living in Oklahoma, seems, however, to indicate that the maize from the Southeastern States tends to have more knobs on its chromosomes than does the maize of the Indian tribes of the North. In general the maize of the Oklahoma Indians seems to be a blend of that from the Indians who lived north, east, and southeast of this central region.

Only in the maize from the Pawnee Indians of Oklahoma is there indication of contamination with that of the Indian tribes to the Southwest. A few plants in the author's collection coming from the Pawnee Indians were found to have the chromosomes generally marked by knobs. These knobs are so similar in number and size to the knobs on maize strains of the Southwest and the general morphological features of the chromosomes of these few plants are so like the chromosomes of the maize of adjacent New Mexican Indians that a relationship is suggested.

Mixing similar to that for the maize of the Oklahoma Indians seems to have begun in the maize of the Ute, Zuni and Tewa, and Mescalero Apache Indians, but in these latter tribes it is the maize of the North and of the Southwest that have recently been brought together in the same tribes. Only in the maize from the Mescalero Apache is there indication that the mixing has been followed by hybridization giving strains with the number of knobs per plant intermediate between northern and southwestern strains. The view that there has been a mixing of the maize from the northern with that from the southwestern Indians is supported in one instance by anthropologists who consider the Zuni and Tewa Indians to be a mixture of Pueblo and some tribe of northern Indians.

The seed from the Walapai Indians came from the reservation on the west border of Arizona and that from the Mission Indians from the Pala Reservation in southwest California. The strains from both tribes have relatively few knobs on their chromosomes and hence differ widely from the varieties grown by their adjacent neighbors on the east.

Insofar as the number of knobs per plant on the chromosomes of maize from various Indian tribes can be considered as indicative of relationship of the maize grown by these Indians, the author would group the various strains into (1) the southwestern group with knobs prevalent, (2) the northern group with knobs rare, and (3) the southeastern group with relatively few knobs, although more than were found on the chromosomes of the northern group.

The author's interpretation of the data supplied by the maize of the Walapai and Mission Indians of the Southwest is that they have

chromosomes with morphological characters more like the maize of the neighboring tribes to the north than like that of tribes to the east.

To visualize more clearly, from a geographical standpoint, the data just discussed, the graph of figure 13 has been prepared. In this the number of knobs per plant for the maize of each State has been averaged, though occasionally two adjacent States are considered together. This graph shows clearly the prevalence of knobs on the chromosomes

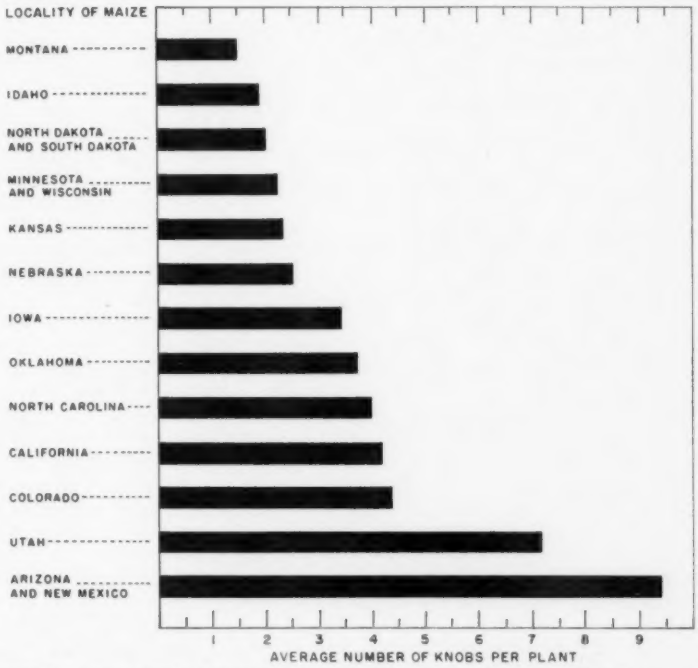

FIGURE 13.—Average number of knobs per plant on the chromosomes of maize from 16 States represented by 13 bars.

in the maize strains from Arizona and New Mexico, and the small number of knobs from the Northern States.

The maize strains of the eastern Indian tribes and those of the tribes from the North have been so jumbled with each other in their Oklahoma location that they show only in a general way a slightly greater prevalence of knobs on their chromosomes than the more isolated northern maize. The author feels, however, after a preliminary study of maize from the West Indies, that there is a resemblance between the chromosomes of the maize from these islands and those of the maize grown by the southeastern Indians. The possibility is thus suggested that maize may have been introduced into the United States through two sources—Mexico and Florida or the Carolinas.

This general study of the knobs on the chromosomes of strains grown by the Indians, combined with a previous study (*3*) of the

chromosomes of the nearer maize relatives, suggests to the author that knobs are a characteristic feature of the chromosomes of maize and its relatives growing in their native habitats. Strains of maize found farthest from Central America and Mexico have lost many of the knobs that mark so prominently the chromosomes of maize and its relatives growing in those regions.

ABNORMALITIES

Those maize strains in which the number of knobs on the chromosomes is highest, have, as one would expect, knobs at positions not previously recorded. It was also in these strains having many knobs that the author found abnormal types of chromosome X. Abnormal types were first seen in an annual teosinte from Mexico and have already been described by the author (*3*). Twenty-three plants with the long form of the tenth chromosome were found in the maize strains from the Navajo, Pueblo, Mescalero Apache, and Ute Indians. In one substrain of maize from the Navajo Indians the tenth chromosome was normal in length but had a prominent chromomere near the end of the long arm at a position usually marked by an enlargement in the longer abnormal form of this chromosome.

A study of the association between the number of knobs on the chromosomes and the occurrence of B-type chromosomes was made. Dividing the data into two groups, one containing those plants with more than 7 knobs and the other those plants with less than 7 knobs, showed that in the 145 plants with more than 7 knobs only 9, or 6.2 percent, carried B-type chromosomes, whereas in the 286 plants with less than 7 knobs 57, or 20 percent, carried B-type chromosomes. This greater prevalence of B-type chromosomes in plants with few knobs was illustrated further when after the subdivision and examination of the maize strains from the Mescalero Apache, the Zuni and Tewa, Ute, and Pawnee tribes, it was found that in every case the plants with B-type chromosomes were plants with few knobs on their chromosomes.

With one exception B-type chromosomes in all plants appeared identical and were marked by darkly stained areas similar to those pictured by McClintock (*6*). In the Pawnee strain, however, diminutive B-type chromosomes, similar to those described by Randolph (*10*), were found. These diminutive chromosomes were frequently associated with the normal B-type chromosomes, clearly proving their relationship. When, however, they were found unassociated with the normal B-type chromosomes it could be seen that each was a spindle fiber attachment and a short thread three or four chromomeres long.

SUMMARY

The presence of knobs on the threadlike paired chromosomes at prophase of the first reduction division of the pollen mother cells of maize is one of the most useful morphological characters in identifying the chromosomes.

A survey of the maize strains from 33 Indian tribes was successful in adding a few more prominent knobs to those already known and in the discovery of a form of the tenth chromosome previously unknown in maize.

The data show very few knobs on the chromosomes from the maize of the northern Indian tribes, a slightly higher number of knobs on the chromosomes of maize from the Indian tribes of the Southeast, and many knobs on the chromosomes of practically all the maize from Arizona and New Mexico.

The number of knobs on the chromosomes of the maize of the Indians of the United States increases as the tribes approach Mexico, where the much-knobbed chromosomes of maize and maize relatives occur.

The number of knobs on the chromosomes of a strain of Indian corn may give a clue to the geographical origin of the strain.

B-type chromosomes, although prevalent in most strains studied, were found more frequently in plants with less than seven knobs than in plants with more than seven knobs on their chromosomes.

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ESTABLISHMENT AND SPREAD OF MOLDS AND BACTERIA ON COTTON ROOTS BY SEED AND SEEDLING INOCULATION[1]

By MARIE B. MORROW, *special agent*, JAMES L. ROBERTS, formerly *agent, Division of Soil Microbiology*, JAMES E. ADAMS, *associate soil technologist*, H. V. JORDAN *associate soil technologist*, and PAUL GUEST, formerly *agent, Division of Soil Fertility Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Investigations conducted in the Blackland prairie section of Texas by Jordan, Dawson, Skinner, and Hunter (*5*),[2] of the Division of Soil Fertility Investigations, Bureau of Plant Industry, indicated a need for a study of the changes in the microflora attending certain crop practices. These and subsequent projects, both field and laboratory, carried out in cooperation with the Division of Soil Microbiology, Bureau of Plant Industry, and the Department of Botany and Bacteriology of the University of Texas, have indicated the desirability of investigating the relation of native and other organisms to the soil-borne pathogen *Phymatotrichum omnivorum* (Shear) Duggar.

The general relations of the pathogen to the cotton plant are well described in the literature. The results of various investigations of biological control of soil pathogens have been summarized by Waksman (*9*). The first step in the practical application of biological control is the establishment of desirable organisms in the soil under field conditions. That soil inoculation has been successful with the rhizobia (*1*) is well known, and establishment is inferred in the work reported where biological control of soil pathogens has been accomplished.

This paper briefly reports the results obtained in the analysis of plant roots and adhering soil for introduced bacteria and molds following the inoculation of cotton seed and seedlings. It appeared desirable to conduct the experiment in a field infested with root rot so that both infected and healthy plants would be available for comparison. The field plan was not sufficiently comprehensive to permit interpretation with assurance of possible indications of biological control due to the introduced organisms. The immediate objective of the work was to determine whether or not the bacteria and molds inoculated upon seed and seedlings are in this way established in the soil.

PROCEDURE AND MATERIAL

FIELD PLOTS

The experimental field was divided into three sections, in each of which inoculation was accomplished by a different method, designated as treatment 1, 2, or 3. Treatment 1 included eighteen 300-foot rows; the other treatments, eighteen 100-foot rows each.

[1] Received for publication August 20, 1937; issued March 1938.
[2] Reference is made by number (italic) to Literature Cited, p. 207.

TEST ORGANISMS

Three molds and two bacteria antagonistic to *Phymatotrichum omnivorum* in laboratory culture were selected.

Aspergillus luchuensis.—This organism was isolated from Texas soil, where heavy infestation had prevailed for years but from which root rot had been retreating for several seasons. It was selected in preference to other black aspergilli because its purple-brown color and single row of sterigmata facilitate identification.

Pseudomonas fluorescens.[3]—This strain was isolated from Texas soil about live, healthy roots of cotton in soil that had been continuously free from root rot.

Penicillium luteum.[3]—This fungus was isolated from roots of green plants taken from an area which, insofar as records reveal, had never been infested with root rot. Culture characteristics make fairly certain the identification of the fungus when reisolated. Results of Wehmer (*10*) and others (*6*) further justify its inclusion.

Achromobacter radiobacter.[3]—The culture used was obtained from the University of Wisconsin. Because of its association with rhizobia of legume roots, this form has long held the interest of soil microbiologists.

Trichoderma lignorum.[3]—The culture used was obtained from the same area as the *Aspergillus*. In view of the interesting observations of Weindling (*11*, *12*, *13*) and others on its parasitism of soil-borne pathogens, this fungus was selected.

INOCULATION AND PLANTING

Treatment 1.—Mebane cotton seeds were soaked for 1 hour in 10-day cultures of the respective organisms in nutrient broth. (The molds were cultured in glucose-nutrient broth.) Growth masses and pellicles were well broken by shaking them with glass beads. When the seeds were taken from the culture, as much of the liquid as possible was removed by squeezing lightly, and the moist masses of seeds were spread over paper and partly dried. Treated in this way the seeds were dry enough to pass through the planter, but moist enough to carry the inoculum. Two 300-foot rows for each of the five organisms, alternating with two control rows of uninoculated seed (treated with sterile broth), were planted.[4]

Treatments 2 and 3.—Untreated seeds were planted for subsequent seedling inoculation.[4] In treatment 2,[4] seedlings were inoculated with heavy broth cultures of the respective organisms, sterile broth being added to the control rows. The roots were exposed by a hand trowel and the culture (25–50 cc) poured about the root crown, after which the soil was carefully replaced. For treatment 3,[5] 10-day cultures inoculated on potato-dextrose agar at pH 6.5 (2.5-cm cubes) were introduced as in treatment 2, sterile agar being placed in the control rows.

LABORATORY PROCEDURE

Samples selected for analysis[6] were lateral roots and taproots from both single plants and groups of plants and, in some cases, soil adjacent

[3] This species name designates a great group and is used here in the sense of an aggregate of strains.
[4] Seed planted April 17, 1936.
[5] Treatment introduced June 15, 1936.
[6] Collection of samples, July 9 to August 8, 1936.

to the roots. The root or soil was carefully exposed and removed to sterile soil cans. The samples were usually analyzed within 24 hours after collection; during any delay, however, they were refrigerated at 6.0° C.

Each sample was weighed and then washed thoroughly in sterile tap water by being shaken with beads in screw-cap prescription bottles. Three dilutions of the washings were made, and each dilution was plated in quintuplicate on two different media. While efforts were directed chiefly toward a qualitative analysis of the flora, quantitative analysis was roughly made where possible.

The molds were recovered from Waksman's (*8*) acid-glucose agar. A modified sodium albuminate agar, used in attempts to recover *Pseudomonas fluorescens* and *Achromobacter radiobacter*, was compounded as follows: Agar, 12.5 g; mannitol, 5 g; glucose, 0.5 g; dipotassium phosphate (K_2HPO_4), 0.5 g; magnesium sulphate ($MgSO_4$), 0.2 g; ferric sulphate ($Fe_2(SO_4)_3$), trace; egg albumen, 0.25 g; peptone, 2.0 g; yeast extract, 25 cc; and distilled water, 975 cc.

After 24 and 48 hours' incubation at 28° C., the plates were studied for the occurrence of *Pseudomonas fluorescens;* after 4 days, for *Achromobacter radiobacter* and the molds. Any colony suspected of being from an inoculated organism was picked to a suitable medium (Waksman's medium for molds; albuminate medium for bacteria), purified, and identified by the usual routine morphological and cultural study. Agglutination reaction in immune sera was used as an aid in identifying *Ach. radiobacter.*

RECOVERY OF ORGANISMS FROM INOCULATED AND NONINOCULATED PLANTS

Throughout the investigation, the recovery of *Achromobacter radiobacter* and *Pseudomonas fluorescens* was considered uncertain. In the case of *Ach. radiobacter* a large number of colonies were isolated, purified, and studied as to fermentation characteristics, physiological tests, and reactions to known *Ach. radiobacter* antiserum. Except in a few cases the results were negative or doubtful. The agglutination reaction, which was considered the most reliable test, eliminated a majority of suspected cultures. Those cases considered positive did not agglutinate to full titer. Also, it has been almost impossible to establish with certainty that many of the *Ps. fluorescens* isolations were actually reisolations of the inoculated cells, because of the expected distribution of this organism in normal soil.

On the other hand, the results would indicate that the molds which were introduced about the root systems were in many cases established there. These results are summarized in table 1. *Aspergillus luchuensis* was found on both healthy and diseased roots in sufficiently greater numbers where inoculated to validate this conclusion. On the other hand, it was never recovered from soil and roots in areas away from the experimental plots. The recovery in a majority of samples analyzed of a form so consistently absent from soil not inoculated with it and from plants not in contact with inoculation would seem to point to successful establishment during the period of observation.

TABLE 1.—*Recovery of test organisms from inoculated and noninoculated plants*

Test organism used for inoculation, and treatment	Samples studied [1]		Root samples analyzed [2]		Healthy plants [3]		Diseased plants [3]		Samples from treatment [3]—					
									1		2		3	
	Total	Cases of recovery	Total	Cases of recovery	Total	Cases of recovery	Total	Cases of recovery	Total	Cases of recovery	Total	Cases of recovery	Total	Cases of recovery
Aspergillus luchuensis:	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Not inoculated	106	6	86	6	43	0	43	6	22	0	50	3	22	2
Inoculated	16	7	16	7	8	2	8	5	2	2	12	4	2	1
Odds [4]	1 : 150,000		1 : 16,000		1 : 2,000		1 : 375,000		1 : 16,000		1 : 370		1 : 6.5	
Trichoderma lignorum:														
Not inoculated	66	4	66	4	33	2	33	2	12	1	40	2	12	1
Inoculated	64	24	44	18	23	7	21	11	12	4	20	9	12	5
Odds [4]	1 : 150,000		1 : 150,000		1 : 80		1 : 16,000		1 : 80		1 : 16,000		1 : 80	
Penicillium luteum.														
Not inoculated	114	44	94	38	48	23	46	17	22	9	50	22	22	9
Inoculated	16	15	16	15	8	7	8	8	2	2	12	11	2	2
Odds [4]	1 : 16,000		1 : 16,000		1 : 20		1 : 2,000		1 : 6.5		1 : 370		1 : 6.5	

[1] Samples included soil and roots, diseased and healthy, single and composite.
[2] Single and composite, from all 3 treatments.
[3] Comparison of recovery from various treatments, both healthy and diseased plants, single and composite samples, from lateral roots only.
[4] Odds against random distribution (7).

Trichoderma lignorum seems to have been established on both healthy and diseased roots in treatments 2 and 3. From the more or less consistent recovery of this form and its equally noticeable absence from plants not inoculated, it would appear that *Trichoderma* has been recoverable over a period of months from plots in contact with inoculum.

Penicillium luteum, although found in normal Texas soil, which complicates interpretation, was recovered in an appreciably greater percentage of cases where inoculated than where it was not introduced. This was especially noticeable on diseased plants and in treatment 2. Although sporadic occurrence is frequent, the evidence herein presented points to a fairly consistent occurrence of *P. luteum* where inoculated.

No detailed study of the spread of molds through soil was made. The findings presented confirm those of Garrett (*3*) in showing that molds are capable of following the surfaces of plant roots as they spread through the soil.

DISTRIBUTION OF INTRODUCED ORGANISMS OVER ROOT SYSTEMS OF INDIVIDUAL PLANTS

The consistent recovery of *Aspergillus luchuensis* and *Trichoderma lignorum* from growing cotton roots suggested the need for further investigation to determine how much of the root system is infected following seed and seedling inoculation. The following procedure was designed to demonstrate the distribution of the introduced organisms over the root systems of the inoculated plants.

SAMPLING

The soil in which two plants, one healthy and one diseased, stood side by side in the same row, was excavated so that the root systems were exposed. A sketch of the root system was drawn to scale, and approximately 10 samples were taken from each root system; each sample was given a number corresponding to a number on the sketch. Samples were 3-inch portions taken from the tip, middle, and base of lateral roots and taproots. Each sample was placed in a tightly stoppered, sterile test tube, and refrigerated until analyzed.

A pair of root systems, one each from a healthy and a diseased plant, were excavated in each of the following locations:

Treatment 2, *Aspergillus luchuensis* rows.
Treatment 2, *Pseudomonas fluorescens* rows.
Treatment 2, *Penicillium luteum* rows.
Treatment 2, *Achromobacter radiobacter* rows.
Treatment 2, *Trichoderma lignorum* rows.
Treatment 1, *Aspergillus luchuensis* rows.
Treatment 1, *Trichoderma lignorum* rows.
Field infested with root rot, 200 feet from the experimental plot.

PLATING

Plates of Waksman's (*8*) and Jordan's (*4*) selective media for *Ps. fluorescens*, and yeast water-mannitol-Congo red agar, selective for *Ach. radiobacter* (*2*), were poured and the agar surfaces thoroughly dried. The root samples were cut into three equal parts and placed respectively on the three media, being pressed lightly to hold them firmly on the surface of the medium. The plates were incubated at 28° C., and daily observations were made.

RESULTS

In treatment 1, *Trichoderma lignorum* was recovered where inoculated in 3 of the 20 samples from two plants studied, twice on healthy roots and once on diseased roots. These results are summarized in table 2. On 20 samples from two plants not inoculated, the organism was never found. In the same treatment, *Aspergillus luchuensis* was

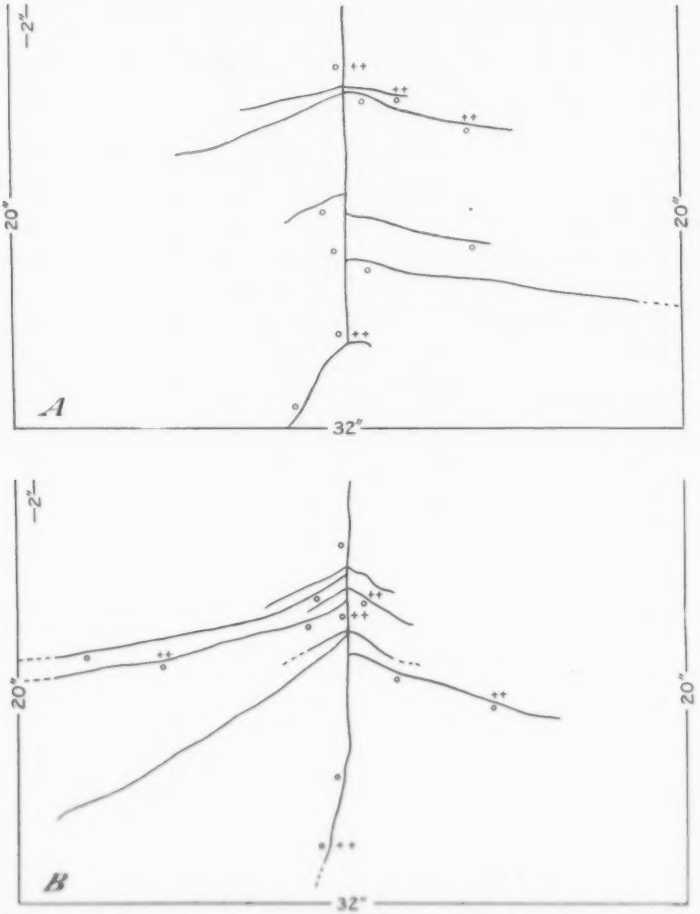

FIGURE 1.—*A*, Root of diseased cotton plant inoculated with *Aspergillus luchuensis*; treatment 1. *B*, Root of healthy cotton plant inoculated with *A. luchuensis*; treatment 2. ○, Locations sampled; ++, *A. luchuensis* recovered.

isolated six times from the 20 samples taken from two inoculated plants, but it was not found on plants not receiving inoculum.

In treatment 2, *Trichoderma* was recovered from 8 samples of the 19 taken from two plants inoculated with *Trichoderma*, and twice on 80 samples from eight plants not inoculated. *Aspergillus* was isolated

TABLE 2.—*Summary of results*

Location, treatment, and condition of plants	Cases of recovery of—	
	Trichoderma lignorum	*Aspergillus luchuensis*
	Number	*Number*
Treatment 1, rows inoculated with—		
Trichoderma lignorum:		
Healthy	2 out of 10	0 out of 10
Diseased	1 out of 10	0 out of 10
Total	3 out of 20	0 out of 20
Aspergillus luchuensis:		
Healthy	0 out of 10	3 out of 10
Diseased	0 out of 10	3 out of 10
Total	0 out of 20	6 out of 20
Treatment 2, rows inoculated with—		
Trichoderma lignorum:		
Healthy	5 out of 10	0 out of 10
Diseased	3 out of 9	0 out of 10
Total	8 out of 19	0 out of 20
Aspergillus luchuensis:		
Healthy	0 out of 10	5 out of 10
Diseased	0 out of 10	2 out of 10
Total	0 out of 20	7 out of 20
Penicillium luteum:		
Healthy	1 out of 10	0 out of 10
Diseased	0 out of 10	0 out of 10
Total	1 out of 20	0 out of 20
Pseudomonas fluorescens:		
Healthy	0 out of 10	0 out of 10
Diseased	0 out of 10	0 out of 10
Total	0 out of 20	0 out of 20
Achromobacter radiobacter:		
Healthy	0 out of 10	0 out of 10
Diseased	1 out of 10	0 out of 10
Total	1 out of 20	0 out of 20
Field 200 feet from experimental plot:		
Healthy	0 out of 10	1 out of 10
Diseased	0 out of 10	0 out of 10
Total	0 out of 20	1 out of 20
Total:		
Trichoderma lignorum inoculated rows:		
Healthy	7 out of 20	
Diseased	4 out of 19	
Total	11 out of 39	
Trichoderma lignorum noninoculated rows:		
Healthy	1 out of 60	
Diseased	1 out of 60	
Total	2 out of 120	
Odds against random distribution	1:200,000	
Total:		
Aspergillus luchuensis inoculated rows:		
Healthy		8 out of 20
Diseased		5 out of 20
Total		13 out of 40
Aspergillus luchuensis noninoculated rows:		
Healthy		1 out of 60
Diseased		0 out of 60
Total		1 out of 120
Odds against random distribution		1:200,000

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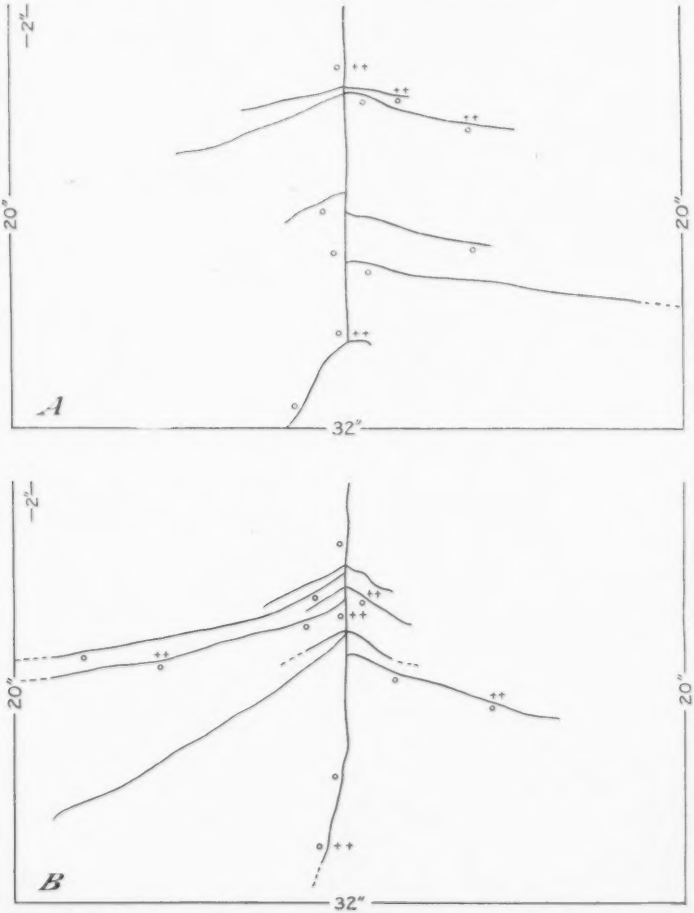

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Trichoderma lignorum:		
Healthy	5 out of 10	0 out of 10
Diseased	3 out of 9	0 out of 10
Total	8 out of 19	0 out of 20
Aspergillus luchuensis:		
Healthy	0 out of 10	5 out of 10
Diseased	0 out of 10	2 out of 10
Total	0 out of 20	7 out of 20
Penicillium luteum:		
Healthy	1 out of 10	0 out of 10
Diseased	0 out of 10	0 out of 10
Total	1 out of 20	0 out of 20
Pseudomonas fluorescens:		
Healthy	0 out of 10	0 out of 10
Diseased	0 out of 10	0 out of 10
Total	0 out of 20	0 out of 20
Achromobacter radiobacter:		
Healthy	0 out of 10	0 out of 10
Diseased	1 out of 10	0 out of 10
Total	1 out of 20	0 out of 20
Field 200 feet from experimental plot:		
Healthy	0 out of 10	1 out of 10
Diseased	0 out of 10	0 out of 10
Total	0 out of 20	1 out of 20
Total:		
Trichoderma lignorum inoculated rows:		
Healthy	7 out of 20	----------
Diseased	4 out of 19	----------
Total	11 out of 39	----------
Trichoderma lignorum noninoculated rows:		
Healthy	1 out of 60	----------
Diseased	1 out of 60	----------
Total	2 out of 120	----------
Odds against random distribution	1:200, 000	----------
Total:		
Aspergillus luchuensis inoculated rows:		
Healthy	----------	8 out of 20
Diseased	----------	5 out of 20
Total	----------	13 out of 40
Aspergillus luchuensis noninoculated rows:		
Healthy	----------	1 out of 60
Diseased	----------	0 out of 60
Total	----------	1 out of 120
Odds against random distribution	----------	1:200, 000

from 7 of the 20 samples from two *Aspergillus*-inoculated plants, and was not found in 80 samples from eight plants not in contact with inoculum. These results would seem to confirm the conclusion that the molds *Trichoderma lignorum* and *Aspergillus luchuensis* may be

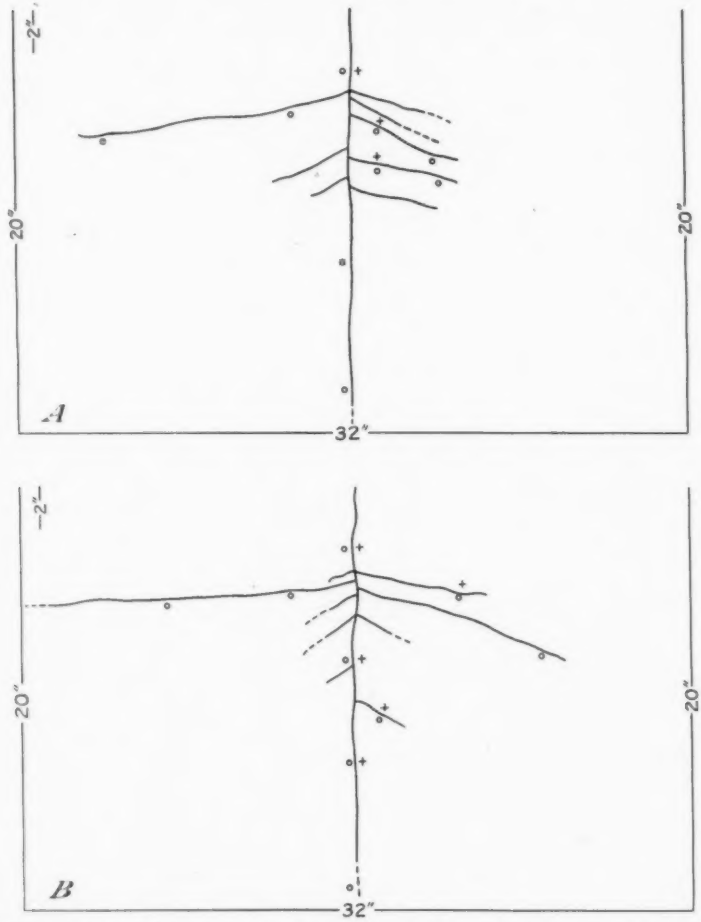

FIGURE 2.—*A*, Root of diseased cotton plant inoculated with *Trichoderma lignorum;* treatment 2. *B*, Root of healthy cotton plant inoculated with *T. lignorum;* treatment 2. o. Locations sampled; +, *T. lignorum* recovered.

found on the living root systems and can be recovered from inoculated plants in from 6 weeks to 4 months after seed and seedling inoculation.

Penicillium luteum was isolated in a large number of cases, but it was also found where it had not been introduced. Because of this it is difficult to draw any conclusions concerning its occurrence or distribution. As in the previous part of the investigation, the

recovery of *Achromobacter radiobacter* and *Pseudomonas fluorescens* was uncertain.

Having determined those root samples in which inoculated organisms occurred, it has been possible to indicate, on the diagrams constructed to show sampling locations, the distribution of the inoculated

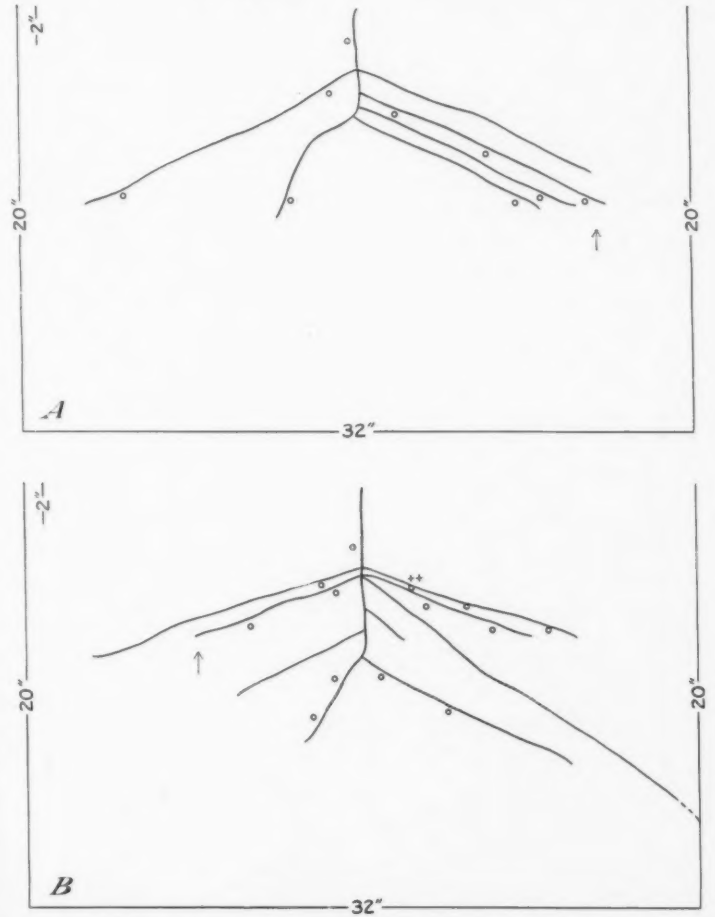

FIGURE 3.—Distribution of *Trichoderma lignorum* and *Aspergillus luchuensis* on root of (*A*) diseased cotton plant and (*B*) healthy plant. o. Locations sampled; ++, *A. luchuensis* recovered. Arrows indicate diseased root of healthy plant adjacent to diseased plant.

forms on a single root system. Six such diagrams are presented in figures 1 to 4.

The diagrams shown in figure 3 are particularly interesting, since they represent growing root systems of a diseased and of a previously healthy plant side by side in the same row and show very strikingly the beginning of infection on one of the lateral roots of a healthy plant

in contact with the infected roots of a diseased plant. No other root on the healthy plant showed symptoms of root rot; and this root showed symptoms only at the tip, where it was in direct contact with the infected root of the diseased plant.

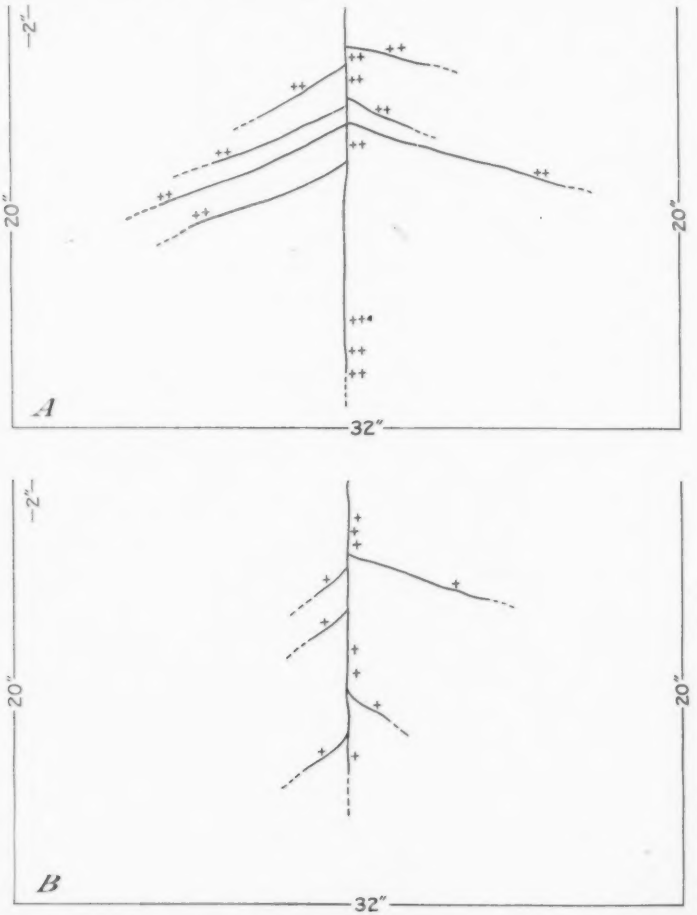

FIGURE 4.—Hypothetical distribution on cotton root of *Aspergillus luchuensis* (++) (*A*) and *Trichoderma lignorum* (+) (*B*).

Shown in figure 4 are all the locations pertaining to *Aspergillus luchuensis* and *Trichoderma lignorum*. This figure represents hypothetical plants with root systems drawn through every point where the respective organisms were found. It is interesting to note that in general *Trichoderma* was recovered only on or near the taproot, while *A. luchuensis* was well distributed over both the taproot and laterals. In view of the fact that the inoculum was placed about the taproot at

a depth of only 4 inches and that these molds have been recovered to a depth of 13 and 17 inches, respectively, for *Trichoderma* and *Aspergillus*, and that the latter organism shows lateral spread from the taproot of some 9 inches, it would seem that the introduced molds have spread on the growing roots, whether or not they have spread through soil free from root material.

SUMMARY

A study of plants from a field infested with *Phymatotrichum omnivorum* (Shear) Duggar has demonstrated that organisms introduced by inoculated cotton seed and by inoculum furnished to the cotton seedling have been established in the rhizosphere of the plant. This conclusion is based upon recovery of the introduced organisms in appreciably greater numbers from inoculated plants than from those not receiving inoculum.

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THE COMPARATIVE VALUE OF COD-LIVER OIL ALONE AND IN COMBINATION WITH IRRADIATED ERGOSTEROL AS A SOURCE OF VITAMIN D FOR CONFINED LAYING HENS [1]

By RALPH B. NESTLER

Junior biologist, Animal Nutrition Division, Bureau of Animal Industry, United States Department of Agriculture

SURVEY OF LITERATURE

Cod-liver oil has been used for many centuries as an antirachitic agent (*10*).[2] In 1922 McCollum, Simmonds, Shipley, and Park (*24, p. 7*) published an account of a newly discovered factor in cod-liver oil, separate from vitamin A, "which exerts a directive influence on the bone development and enables animals to develop on an inadequate supply of calcium much better than they could otherwise do." Four years later Holmes, Brown, Smith, Treadwell, and Whitelock (*18*) pointed out that cod-liver oil in the diet of laying hens increased egg production and hatchability. That the newly discovered vitamin D was a major factor in their results can be gathered from the work of Hughes, Payne, and Latshaw (*20*), who had previously obtained the same results with ultraviolet irradiation.

In 1924 Steenbock and Black (*31*) found that diets for rats could be made antirachitic and growth promoting by irradiation with a quartz mercury-vapor lamp. In the same year Hess (*13*) and Hess and Weinstock (*15*) activated linseed oil and cottonseed oil by means of irradiation. Either of these activated oils when added to a rickets-producing diet was able to protect rats from rickets. Hess and Weinstock also found that by irradiating cod-liver oil they could enhance its antirachitic value.

Since the unsaponifiable portion of fat contains a large quantity of cholesterol, for a time many research workers considered cholesterol as the precursor of vitamin D. However, Heilbron, Kamm, and Morton (*12*), Hess and Windaus (*16*), and Bills, Honeywell, and MacNair (*6*) demonstrated that in the nutrition of rats, the vitamin D precursor is ergosterol, which is present as a minute impurity in the cholesterol. From this irradiated sterol a crystalline "synthetic" vitamin D was finally isolated. Dissolved in corn oil, irradiated ergosterol is being used extensively as a source of vitamin D.

In the nutrition of rats, Russell, Taylor, and Wilcox (*30*) found that irradiated ergosterol was as efficacious in preventing rickets as cod-liver oil when the two were fed in the approximate equivalent rat-unit dosage. However, in human nutrition, De Sanctis and Craig (*8*) and Barnes, Brady, and James (*1*) concluded that irradiated ergosterol was not so satisfactory an agent in the prevention of rickets as cod-liver oil in the approximate equivalent rat-unit dosage. Holmes (*17, p. 214*), after making a comprehensive review of the

[1] Received for publication October 9, 1937; issued March, 1938.

[2] Reference is made by number (italic) to Literature Cited, p. 218.

literature, decided that as an antirachitic agent "either vitamin D as it occurs in cod-liver oil possesses properties not present in irradiated ergosterol, or that the vitamin A or some other substance in cod-liver oil enhances the antirachitic vitamin in the oil * * *." In the bone development and growth of chicks, Mussehl and Ackerson (*27*), Massengale and Bills (*25*), Bethke, Record, and Kennard (*2*), and Massengale and Nussmeier (*26*) also found that activated ergosterol was much less effective than the rat-equivalent quantity of cod-liver oil. Bethke and associates estimated that 15 to 20 times as many rat units of vitamin D per unit of feed, in the form of irradiated ergosterol, is required for normal bone formation, as in the form of cod-liver oil. Finally, in the feeding of mature hens for optimum egg production and hatchability, Branion and Smith (*7*), Russell, Taylor, and Wilcox (*29*), Bethke, Record, Kick, and Kennard (*3*), and Titus and Nestler (*33*) showed that irradiated ergosterol gave poorer results than the rat-equivalent dosage of cod-liver oil.

Titus and Nestler found that cod-liver oil fed at high levels had a markedly deleterious effect on both egg production and hatchability. They suggested that the optimum quantity of cod-liver oil for strictly confined laying hens is between 1 and 2 percent of the diet, and that when irradiated ergosterol is used as a source of vitamin D, 10 to 20 times as much of this vitamin (as measured by assays with rats) must be fed.

Barnes and associates (*1*), in their observations on humans, found that there was no evidence of superiority among a group of children receiving a mixture of cod-liver oil and irradiated ergosterol as compared with a group given cod-liver oil only. Bethke and associates (*2*) concluded from their work with chicks that a mixture of cod-liver oil and irradiated ergosterol is less effective than the rat-equivalent quantity of cod-liver oil only for proper bone calcification.

Holmes and Pigott (*19*) determined on rats the antirachitic activity of six samples representing five brands of irradiated ergosterol 100 D and found that these samples did not possess uniform antirachitic value. A sample of irradiated ergosterol 100 D that possessed the highest activity of those listed had less than 60 times the antirachitic activity of the best cod-liver oil tested.

Rider, Sperti, Goode, and Cassidy (*28, p. 456*), after making a comprehensive review of the literature, suggested that—

> the expected and the actual clinical potencies of irradiated ergosterol may have been accounted for in part by the now overwhelmingly evident fact that irradiation of ergosterol with the full ultraviolet of the quartz mercury arc produces a multiplicity of products. Some of these products are known to be inactive physiologically and others to be toxic without appreciable antirachitic activity.

These workers found that the discrepancies between the activities of antirachitic agents in rats and chickens are to some extent paralleled by similar discrepancies between their activities in rats and children. In the absence of vitamin D the chicken develops rickets in spite of an optimum calcium and phosphorus ratio and content in the diet. To produce rickets in the rat the diet must be definitely unbalanced with respect to these two constituents. Hess, Lewis, and Rivkin (*14, p. 1888*) make the observation that—

> there is an essential difference in the pathogenesis of rickets in the rat and in infants, that the former is regularly brought about simply by a lack of phosphorus in the diet, whereas rickets never comes about in infants as a result of such a deficiency * * *.

When Bills and associates (*6*) in 1928 activated by ultraviolet irradiation the ergosterol that they obtained from cholesterol, they noted that they could also activate the ergosterol-free cholesterol by the same irradiation. The following year Koch, Koch, and Lemon (*22*) and Koch, Koch, and Ragins (*23*) concluded that provitamin D activity is not limited to ergosterol, but that it may be a general property in varying degrees of various sterols or certain forms of these sterols. More recently Waddell (*34*), Koch and Koch (*21*), Hathaway and Lobb (*11*), and Bethke, Record, and Wilder (*4*) found that in chicken nutrition the vitamin D of irradiated, heated, and purified cholesterol has properties resembling more closely the natural vitamin D of cod-liver oil than has the vitamin D of irradiated ergosterol. In 1937, Bills (*5*) pointed out that at least eight forms of vitamin D have been artificially prepared, and at least two forms occur in fish oils.

In view of the fact that the chicken is less sensitive than the rat to the vitamin D produced by irradiating ergosterol, whereas the opposite is true of the vitamin D in cod-liver oil, the question arises: Is a mixture of vitamin D from these two sources more effective in the case of laying hens than the vitamin D from cod-liver oil alone? As a result of an experiment originally planned for a study of the effect of irradiated ergosterol on the transfer of vitamin A to eggs (DeVaney, Titus, and Nestler (*9*)), data have been accumulated which throw some light on this question. The present paper deals with the writer's analysis of these data.

EXPERIMENTAL METHODS AND MATERIALS

The experiment reported in this paper was conducted at the Agricultural Research Center, Beltsville, Md., during the 48-week period beginning August 29, 1933, and ended July 31, 1934. Six pens were used in this study. They were approximately 8 by 11 feet in size and were located on the second floor of a frame building. Ordinary window glass filtered all the sunlight that entered.

In this experiment there were used 108 cross-bred pullets, the progeny of a mating of Rhode Island Red males with Barred Plymouth Rock females. These birds were distributed in the 6 pens at random, 18 to a pen. Two Rhode Island Red cockerels were placed in each pen, but 5 weeks later the poorer one of each pair was removed. To insure uniform fertility the males were transferred from one pen to the next twice a week.

Throughout the experiment the birds were allowed all they would eat of the following all-mash diet:

	Percent
Ground yellow corn	38. 5
Pure wheat bran	18. 8
Rolled oats	11. 6
Alfalfa leaf meal	4. 2
Desiccated meat meal	8. 0
North Atlantic fish meal	7. 0
Dried buttermilk	5. 0
Ground limestone	2. 9
Special steamed bonemeal	3. 0
Anhydrous sodium sulphate	. 5
Sodium chloride	. 5
Total	100. 0

The proximate chemical analysis of this diet was as follows:

	Percent
Moisture	10.0
True protein [1]	19.2
Nonprotein nitrogen compounds	1.7
Ash	10.3
Fat	4.4
Fiber	4.1
Nitrogen-free extract	50.3
Total	100.0

[1] The diet contained 21.5 percent of crude protein.

A phosphorus level of 1.2 percent and a calcium level of 3.0 percent were maintained throughout the experiment.

The following oil mixtures were added to the diet: Mixture of (1) 0.5 percent of corn oil and (2) 1 percent of cod-liver oil for pen 1, 2 percent of cod-liver oil for pen 2, and 8 percent of cod-liver oil for pen 3; and a mixture of (1) 0.5 percent of irradiated ergosterol 160 D and (2) 1 percent of cod-liver oil for pen 1A, 2 percent of cod-liver oil for pen 2A, and 8 percent of cod-liver oil for pen 3A.

The rachitically inert corn oil was used merely to make the fat content of the diets fed in the first three pens correspond with that of the diets fed in the last three pens. On the basis of the conclusion drawn by Titus and Nestler (*33*), 0.5 percent of irradiated ergosterol 160 D may be considered equivalent to 4 percent of cod-liver oil in its vitamin D value to laying hens. Therefore, whereas pens 1, 2, and 3 received 1, 2, and 8 percent of cod-liver oil, respectively, pens 1A, 2A, and 3A received the equivalent of 5, 6, and 12 percent of cod-liver oil, respectively.

The weights of the birds were obtained at the beginning of the experiment and at the end of every 4-week period thereafter. A record was kept of the weight of the feed given to the birds and the weight of the residue at the end of each 4-week period. Four times a day throughout the experiment the birds in the trap nests were removed and the eggs were collected. Every 4 weeks all the sound eggs laid during a period of 10 days were incubated in order to obtain data on fertility and hatchability. During thirty-three 5-day periods, systematically scheduled throughout the year, all eggs were weighed; and during every third 5-day period the eggs, in addition to being weighed, were tested for shell strength by the method of Swenson and James (*32*), as modified by Titus and Nestler (*33*). In this test for eggshell strength a steel ball weighing 3.5282 g was dropped from various heights through a tube on each egg, which was held in place under the end of the tube. During the last half of the experiment studies were made of the weight and thickness of the eggshells, thickness of the membranes of the eggshells, weight and color index of the yolks, and the weight of the whole and thick albumen, from eggs obtained during five 5-day periods 4 weeks apart. The thickness of the eggshells and the thickness of the shell membranes were determined with micrometer calipers immediately after the eggs were broken. The two membranes were measured together. A color scale designed by Dr. Paul F. Sharp, of Cornell University, was used in the study of the color of the egg yolks. The thick albumen was sepa-

rated from the whole albumen by the use of a perforated sieve $4\frac{5}{7}$ inches in diameter, having 64 holes per square inch, each hole being 0.081 inch in diameter.

RESULTS AND DISCUSSION

A summary of the data obtained is presented in table 1, and the statistical significance of the differences found in table 1 is presented in table 2. Most of the data were analyzed by variance analysis.

The birds in pen 1, which received 1 percent of cod-liver oil as the source of vitamin D, made significantly greater gains in live weight between August 29, 1933, and February 13, 1934—the date of maximum gains during the entire experiment—than the birds in pens 1A, 2, or 2A. The differences between the gain in live weight of the birds in pen 1 and the gains of the birds in pens 3 and 3A were large but not statistically significant. Likewise, differences between the gains in live weight of birds in any two of the pens other than pen 1 were not statistically significant.

The birds in pen 1 consumed a greater quantity of feed than any other birds. This fact accounts for their greater gains in live weight. The difference in feed consumption was statistically significant in all cases, except between the birds in pen 1 and those in pen 2A. The diets fed in pens 1A, 2, and 2A all caused significantly greater feed consumption than those fed in pens 3 and 3A. There were no statistically significant differences between the quantities of feed consumed by the birds in pens 2 and 3, which received 2 and 8 percent of cod-liver oil, respectively, as the sole source of vitamin D, and the quantities of feed consumed by the birds in pens 2A and 3A, which received corresponding levels of cod-liver oil mixed with irradiated ergosterol.

The hatchability of the eggs from either pen 1, 1A, 2, or 2A was higher than that of the eggs from either pen 3 or 3A, the difference being statistically very significant. There was no statistically significant difference between the hatchability of the eggs from a pen receiving any particular level of cod-liver oil without irradiated ergosterol and that of eggs from a pen receiving a corresponding level of cod-liver oil with irradiated ergosterol.

The production and the total weight of eggs in either pen 1, 1A, 2, or 2A also were greater than the production and the total weight of eggs in either pen 3 or 3A. However, it will be noted from table 2 that the difference between the egg production in either pen 1 or 1A and that in either pen 3 or 3A was not so statistically significant as the difference between the egg production in either pen 2 or 2A and that in either pen 3 or 3A. In the case of both egg production and the total weight of the eggs produced, there was a statistically greater significant difference between either pen 1, 1A, 2, or 2A and pen 3A than between either pen 1, 1A, 2, or 2A and pen 3. Although there was a tendency for cod-liver oil to give better results than a mixture of cod-liver oil and irradiated ergosterol, none of the differences between the results obtained at the same level of cod-liver-oil intake were statistically significant.

The only statistically significant differences found in the data for the average weight of the eggs was between either pen 1 or 1A and either pen 3 or 3A. The weights of the eggs from the former pens were heavier than those of the eggs from the latter pens.

TABLE 1.—*Effect of cod-liver oil at various levels, fed alone or mixed with irradiated ergosterol, on laying hens and their egg production*[1]

DATA THAT SHOW STATISTICALLY SIGNIFICANT DIFFERENCES[2]

Source of vitamin D	Pen no.	Birds at—		Average gain in live weight Aug. 29, 1933, to Feb. 13, 1934[3]	Average feed consumed per bird per 4-week period[4]	Average hatchability of fertile eggs[5]	Average egg production per bird per 308.5 bird days[6]	Total weight of eggs per bird per 313.8 bird days[7]	Average egg weight	Average yolk weight	Average reading of Sharp's yolk color scale
		Beginning of experiment	End of experiment								
		Number	*Number*	*Grams*	*Kilograms*	*Percent*	*Percent*	*Grams*	*Grams*	*Grams*	
1 percent of cod-liver oil	1	18	11	679.2±53.7	4.27± .10	68.0±4.5	42.11±3.53	7,985±672	58.0±1.0	19.1± .4	62±6
1 percent of cod-liver oil plus 0.5 percent of irradiated ergosterol 160 D	1A	18	14	510.3±47.0	4.00± .10	64.4±4.0	42.98±3.21	7,860±613	58.1± .9	18.5± .4	57±6
2 percent of cod-liver oil	2	18	14	477.4±47.0	4.01± .10	70.8±3.9	45.38±3.31	8,080±631	56.2± .9	18.4± .4	57±6
2 percent of cod-liver oil plus 0.5 percent of irradiated ergosterol 160 D	2A	18	12	481.5±47.0	4.05± .10	71.1±3.8	44.93±3.31	7,925±651	55.7±1.0	18.0± .4	54±6
8 percent of cod-liver oil	3	18	12	568.4±48.4	3.48± .10	39.7±4.7	33.91±3.40	5,700±672	53.7±1.0	16.4± .4	41±6
8 percent of cod-liver oil plus 0.5 percent of irradiated ergosterol 160 D	3A	18	7	590.7±51.8	3.69± .10	36.7±5.7	29.56±3.79	5,075±722	54.8±1.0	16.8± .5	40±8

DATA THAT DO NOT SHOW STATISTICALLY SIGNIFICANT DIFFERENCES [2]

Source of vitamin D	Pen no.	Birds at—		Average loss in live weight Feb. 13, 1934, to July 31, 1934	Average eggshell weight [5]	Average thickness of eggshells	Average strength of eggshells (drop of steel ball)	Average thickness of membrane of eggshells	Average total albumen weight per egg	Average percentage of thick albumen	Average fertility of total eggs set [9]
		Beginning of experiment	End of experiment								
		Number	*Number*	*Grams*	*Grams*	*Millimeters*	*Centimeters*	*Millimeters*	*Grams*	*Percent*	*Percent*
1 percent of cod-liver oil	1	18	11	287.7± 84.0	6.73±0.15	0.319±0.007	15.86±0.38	0.040±0.002	33.6±0.8	60.30±1.35	79.1±6.0
1 percent of cod-liver oil plus 0.5 percent of irradiated ergosterol 160 D	1A	18	14	307.5± 74.5	6.57± .14	.307± .006	15.85± .33	.043± .002	34.1± .7	61.44±1.21	84.4±5.6
2 percent of cod-liver oil	2	18	14	322.9± 74.5	6.75± .14	.326± .006	16.90± .34	.041± .002	32.8± .7	58.83±1.21	78.7±5.2
2 percent of cod-liver oil plus 0.5 percent of irradiated ergosterol 160 D	2A	18	12	250.4± 80.4	6.63± .14	.315± .006	16.18± .36	.042± .002	32.2± .7	57.17±1.21	88.7±5.4
8 percent of cod-liver oil	3	18	12	301.3± 80.4	6.47± .14	.318± .006	16.57± .37	.042± .002	32.0± .7	60.79±1.25	88.0±6.8
8 percent of cod-liver oil plus 0.5 per cent of irradiated ergosterol 160 D	3A	18	7	438.6±105.3	6.25± .19	.324± .009	16.44± .39	.046± .003	32.6±1.0	58.52±1.66	87.5±8.1

[1] In this table standard errors, rather than probable errors, are used.
[2] According to Fisher's *z* test for significance.
[3] Date of maximum gain during the entire experiment.
[4] Average of the average weights of feed consumed.
[5] Weighted average of the percentage of hatchability of the fertile eggs set for the individual birds.
[6] Data for birds laying fewer than 5 eggs during the experiment were eliminated. Rest of data was adjusted by covariance analysis for the number of days each bird lived. The average number of days lived per bird for those birds from which data were used in this analysis was 308.5 out of a possible 336.
[7] Data adjusted by covariance analysis for the number of days each bird lived. The average number of days lived per bird for those birds from which data were used in this analysis was 313.8 out of a possible 336.
[8] Fresh, undried eggshells.
[9] Weighted average of the percentage of fertility of the total eggs set for the individual birds.

TABLE 2.—*Statistical significance of the differences found in table 1*

Sources of vitamin D compared [1]	Pen No.	Degree of significance in the results obtained in the experiment in respect to [2]—							
		Gain in live weight Aug. 29, 1933 to Feb. 13, 1934 [3]	Feed consumed per period of 4 weeks	Hatchability of fertile eggs	Eggs produced per bird	Weight of all eggs produced per bird	Egg weight	Yolk weight	Color of yolks
1 percent cod-liver oil and—	1								
1 percent cod-liver oil+irradiated ergosterol	1A	S	S	N	N	N	N	N	N
2 percent cod-liver oil	2	H	S	N	N	N	N	N	N
2 percent cod-liver oil+irradiated ergosterol	2A	H	N	N	N	N	N	S	N
8 percent cod-liver oil	3	N	H	H	N	S	H	H	H–[4]
8 percent cod-liver oil+irradiated ergosterol	3A	N	H	H	S	H	S	H	S
2 percent cod-liver oil and—	2								
8 percent cod-liver oil	3	N	H	H	S	S	N	H	S
8 percent cod-liver oil+ irradiated ergosterol	3A	N	S	H	H	H	N	S	S–[5]
1 percent cod-liver oil+irradiated ergosterol and—	1A								
8 percent cod-liver oil	3	N	H	H	S–[5]	S	H	H	S
8 percent cod-liver oil+irradiated ergosterol	3A	N	S	H	S	H	H	H	S–[5]
2 percent cod-liver oil+irradiated ergosterol and—	2A								
8 percent cod-liver oil	3	N	H	H	S	S	N	H–[4]	N
8 percent cod-liver oil+irradiated ergosterol	3A	N	H	H	H	H	N	S–[5]	N

[1] The differences are in favor of the first-mentioned source of vitamin D of each comparison.
[2] H=highly significant (odds of at least 99 to 1); S=significant (odds of at least 19 to 1); N=not significant.
[3] Date of maximum gain during the entire experiment.
[4] The odds are slightly less than 99 to 1.
[5] The odds are slightly less than 19 to 1.

The tables show that the birds in pen 1, which received 1 percent of cod-liver oil without irradiated ergosterol, laid eggs with significantly heavier yolks than those in pen 2A, which received 2 percent of cod-liver oil with irradiated ergosterol. The difference in the average weight of the yolks of the eggs from pen 2, which received 2 percent of cod-liver oil without irradiated ergosterol, and those from either pen 3 or 3A, was more significant than the difference in the average weight of the yolks of the eggs from pen 2A and those from either pen 3 or 3A.

The color of the yolks of the eggs from pens 1A, 2A, and 3A had a tendency to be lighter than the yolks of the eggs from pens 1, 2, and 3, but the differences were not statistically significant. Significant differences existed between the color of yolks obtained from either pen 1, 1A, or 2 and either pens 3 or 3A. The difference in yolk color between the eggs from pen 2A and either pen 3 or 3A was not statistically significant.

None of the differences in the loss of live weight of the birds, the weight, thickness, or strength of eggshells, the thickness of the shell membranes, the weight of the whole albumen, the percentage of the thick albumen, and the percentage of the fertility of the eggs, were statistically significant.

The data presented in this paper substantiate the conclusion drawn from previous work of Titus and Nestler (*33*) that excessive percentages of cod-liver oil in the diet of confined laying hens have a markedly deleterious effect on both egg production and hatchability. In the present work 8 percent of cod-liver oil in the diet of laying hens considerably lowered egg production, hatchability, average weight of eggs, total weight of eggs produced, weight of egg yolks, and weight of feed consumed. It also caused the yolks to have a lighter color.

A mixture of one-half percent of irradiated ergosterol 160 D, a quantity about four times that considered by Titus and Nestler (*33*) as necessary for laying birds, and 1, 2, or 8 percent of cod-liver oil, gave practically the same results as the same percentages of cod-liver oil when fed alone. This fact harmonizes with the observations of Barnes, Brady, and James (*1*) on the lack of effectiveness of a mixture of cod-liver oil and irradiated ergosterol in human nutrition, and those of Bethke, Record, and Kennard (*2*) on the lack of effectiveness of such a mixture for proper bone calcification in chicks.

SUMMARY

An experiment was carried on at the Agricultural Research Center at Beltsville, Md., in 1933–34, to compare the efficacy of cod-liver oil fed alone with that obtained by mixing it with irradiated ergosterol, as a source of vitamin D for confined laying hens. Six pens of crossbred pullets, the progeny of a mating of Rhode Island Red males with Barred Plymouth Rock females, were used. The birds were kept in strict confinement without access to direct sunlight. These six pens received the following vitamin D supplement: 1, 2, and 8 percent of cod-liver oil in the first three pens, and 1, 2, and 8 percent of cod-liver oil supplemented in each case by 0.5 percent of irradiated ergosterol 160 D in the last three pens. A phosphorus level of 1.2 percent and a calcium level of 3.0 percent were maintained throughout the experiment.

A mixture of 0.5 percent of irradiated ergosterol 160 D with 1, 2, and 8 percent, respectively, of cod-liver oil in the diet of laying hens showed no superiority over the same quantities of cod-liver oil without the addition of irradiated ergosterol.

The relatively high levels of vitamin D produced by combining cod-liver oil at the 1- and 2-percent levels with about four times the quantity of irradiated ergosterol necessary for laying chickens produced no deleterious effects on the hens or their eggs.

Cod-liver oil at the 8-percent level, whether supplemented with irradiated ergosterol or not, had a markedly deleterious effect on the production, average weight, total weight, and hatchability of the eggs; weight of the yolks; and the consumption of feed. It also caused the color of the yolks to be much lighter than those from birds receiving lower levels of cod-liver oil. However, it had no appreciable effect on the other items studied.

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RESISTANCE OF CHICKEN EMBRYOS TO MECHANICAL DISTURBANCES [1]

By MARLOW W. OLSEN, *assistant poultry husbandman*, and THEODORE C. BYERLY, *senior physiologist, Animal Husbandry Division, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

The effect of mechanical shock on eggs during incubation has long been a matter of speculation. The idea is prevalent that a sudden jar or vibrations of the earth such as are caused by earthquakes, dynamite explosions, claps of thunder, or even the passing of trains, affect hatchability. Little or no concrete evidence has been presented to support or to disprove this idea although reliable information would be valuable from a practical, as well as a scientific, standpoint. If developing embryos are more easily killed at one age than another, the hatcheryman could avoid unnecessary handling of the eggs at a critical time, and thereby increase the chances for improving the hatch.

The very extensive literature on the susceptibility of the embryo and its parts to various external agents has been summarized by Hyman.[2] Most of this literature has to do with the origin of terata and contains the results of qualitative experiments with very young embryos or with eggs subjected to various external forces prior to incubation. The opinion is generally accepted that all harmful agents produce similar effects when applied at similar times to the whole embryo and that they affect those parts of the embryo that are developing most rapidly. Since development in earlier stages usually proceeds more rapidly than in later stages, it is generally held that the younger embryos will be most affected by a given force. However, quantitative data on the susceptibility of the embryo at successive stages of incubation and data for later stages are very meager. In this paper quantitative data are presented showing the resistance of the embryo at different stages of development to various forces applied externally to the egg.

MATERIALS AND METHODS

Eggs obtained from a flock of White Leghorns at the National Agricultural Research Center, Beltsville, Md., in 1934, were used for these experiments. The eggs were carefully selected to eliminate, as far as possible, variations due to the breeding and the management of the adult birds and the storage conditions of the eggs previous to incubation.

All the experimental eggs, including the controls, were incubated in a force-draft cabinet-type machine operated at 99.9° F. with a wet-bulb reading of 85°. The eggs were racked in the trays, large end up, and were turned four times daily.

[1] Received for publication September 24, 1937; issued March, 1938.

[2] HYMAN, L. H. THE METABOLIC GRADIENTS OF VERTEBRATE EMBRYOS. III. THE CHICK. Biol. Bull. 52: 1-38, illus. 1927.

Susceptibility of the embryos, at different stages of development, to mechanical shock was tested by applying force to the egg in different ways: (1) By placing eggs in a shaking machine, (2) by centrifuging, (3) by striking the eggs against the operator's hand, and (4) by placing them near blasts of dynamite. In order that only strong embryos would be used, the eggs were removed from the incubator and candled just prior to treatment by one of the foregoing methods. After treatment they were again placed in the machine, where they were left for 3 days before they were again removed and candled. All eggs containing dead embryos were broken and the embryos classified as to time of death.

EXPERIMENTAL RESULTS

EFFECT OF SHAKING

Each day during incubation, two groups of eggs containing embryos of known ages were placed in a mechanical shaking machine and treated for 7 minutes. This machine was driven by an electric motor

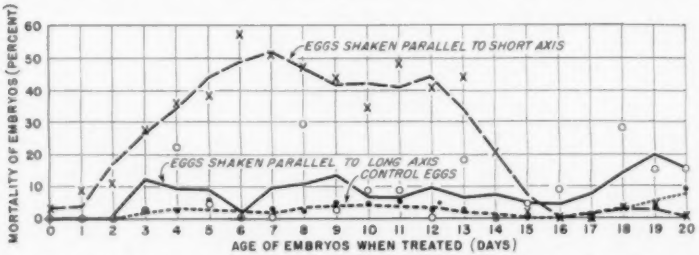
MORTALITY OF EMBRYOS (PERCENT)
60
50
40
30
20
10
0
EGGS SHAKEN PARALLEL TO SHORT AXIS
EGGS SHAKEN PARALLEL TO LONG AXIS
CONTROL EGGS
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
AGE OF EMBRYOS WHEN TREATED (DAYS)

FIGURE 1.—Mortality among embryos in eggs shaken daily for 7 minutes in a mechanical shaker.

at the rate of 229 oscillations per minute through a distance of 3 inches. One group of eggs was so placed in the machine that they were shaken in a direction parallel to the short axis of the egg. The second group was so placed that the direction of force was exerted parallel to the long axis of the egg. During the entire incubation period the test involved 1,252 eggs in the first group, 708 eggs in the second group, and 350 eggs in the control group. The percentage of mortality, showing the effect of this particular type of shock on the embryos at various stages of development, is shown in figure 1

Each point was obtained by dividing the number of deaths which occurred within 72 hours after treatment by the number of live embryos treated. The curves have been smoothed by the three-point method of moving averages. The figure shows that eggs shaken parallel to the short axis of the egg were affected more severely than those shaken parallel to the long axis. In both groups, affected embryos usually died within 72 hours after treatment as a result of rupture of the yolk sac or hemorrhage caused by disruption of the chorio-allantois from the overlying shell membrane. There was little or no delayed mortality and no apparent teratogenic response to treatment at any point in the incubation period. In the eggs shaken parallel to the short axis, mortality attributable to treatment increased rapidly to the fourth day, where it remained within a range

of 30 to 50 percent most of the time until the thirteenth day and then rapidly decreased to zero. In the other treated group, however, the percentage of mortality was rather constant during this period.

EFFECT OF CENTRIFUGING

During each day of the incubation period, two groups of eggs containing embryos of known ages were placed in a centrifuge. One group of eggs, totaling 1,263 at the beginning of this phase of the experiment, was placed in the centrifuge cups blunt end foremost so that the direction of force would be exerted against the large end of the eggs. The second group of eggs, numbering 1,304, was placed pointed end foremost in the centrifuge cups. The eggs were centrifuged for 1 minute, during which time the maximum force exerted on a 2-ounce egg was 1,732 dynes and the average force 710 dynes. After treatment the eggs were removed from the centrifuge and replaced in the incubator, where they remained for 3 days before they were candled and eggs containing dead embryos removed. In

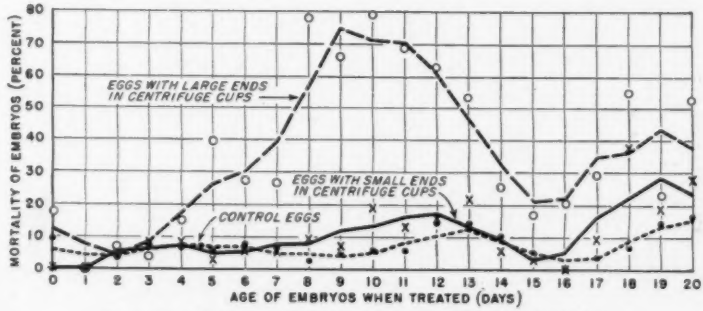

FIGURE 2.—Mortality among embryos in eggs centrifuged for 1 minute.

this phase of the experiment, 850 eggs were used in the control group. The percentage of mortality attributable to treatment on successive days of incubation is shown in figure 2. As in the case of eggs treated in the mechanical shaker, the percentage of mortality was calculated from the number of deaths occurring during a period of 72 hours after treatment. The mortality curves shown in figure 2 have been smoothed by the three-point method of moving averages. The curves are similar to those of eggs that were shaken, in that mortality was much heavier in one of the groups and the heaviest mortality occurred approximately at the same period of incubation.

The effect of treatment in neither group placed in the centrifuge was apparent during the first 3 days of incubation. After that time there was a sharp rise in mortality among embryos in eggs on which force was exerted on the blunt end, the trend line reaching a maximum of 74.0 percent on the ninth day of incubation, gradually receding until it reached 20.5 percent on the fifteenth day, then again rising to an average of about 34.4 percent for the remainder of the incubation period. This same general trend is noticeable but to a far less degree in the group centrifuged with the force exerted on the small end of the egg.

The major cause of death among embryos of both centrifuged groups was found to be broken yolk sacs and ruptured blood vessels of the chorion and the allantois. In these groups of eggs, and especially in the group centrifuged with the large end in the cup, tremulous air cells were produced. Those embryos that survived the treatment showed no ill effects, and in most cases the air cell became fixed again when the chorion and allantois came in contact with the shell membrane. It appears that tremulous air cells are a result of adverse conditions affecting eggs and causing high mortality of the contained embryos and are not the direct cause.

EFFECT OF JARRING

Susceptibility of embryos to mechanical shock was tested by jarring eggs at successive stages of incubation. Each day a group of eggs containing live embryos was removed from the incubator and subdivided into two groups. The blunt ends of one lot of eggs were

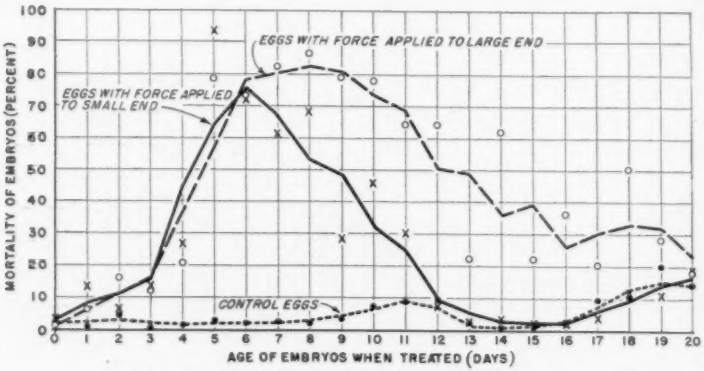

FIGURE 3.—Mortality among embryos in eggs which were jarred against operator's hand.

struck three times against the palm of the operator's hand. The other group was treated in the same manner except that the pointed ends were struck against the operator's hand. In many cases the force was sufficient, especially in the group struck on the blunt end, to produce tremulous air cells in the eggs. During the entire experiment the test involved 1,020 eggs in the first group, 990 in the second group, and 1,163 in the control group. The mortality resulting from the different methods of treatment is shown in figure 3. As in the case of eggs treated in the centrifuge, the points shown on the graph are the percentages of embryos that were killed within 72 hours after treatment. The curves shown have been smoothed by the three-point method of moving averages. It will be noted that the mortality was heavier in the group of embryos in which force was applied on the large end of the egg. This finding is in agreement with that of Knox and Olsen,[3] who found that eggs, when struck on the large end before incubation, failed to hatch as well as either the controls or the group struck on the small end of the egg.

[3] KNOX, C. W., and OLSEN, M. W. THE EFFECT OF TREMULOUS AIR CELLS UPON THE HATCHABILITY OF EGGS. Poultry Sci. 15: 345-348. 1936.

EFFECT OF EXPOSURE TO DYNAMITE BLASTS

Groups of eggs containing embryos of the ages shown in figure 4 were placed near areas where tree stumps were being blasted. The charges of dynamite placed under the stumps ranged from 3 to 28 sticks of 20-percent gelatin dynamite. In this phase of the experiment, 755 eggs were subjected to this type of shock and 623 were used as controls. The former were candled just prior to treatment and then racked, small end uppermost, in an incubator tray and carried to the area where the blasting was being done. The eggs, with the exception of the groups treated on the eighth, ninth, and fourteenth days, were placed within 5 feet of a stump to be blasted and the tray tilted in such a position that the force of the concussion would be exerted on the large end of the egg. To insure against damage from falling objects, the tray of eggs was covered with an empty incubator tray and then was partly buried in the red clay soil. The treatment was so severe that in many cases 20 to 30 percent of the shells of the treated eggs were broken.

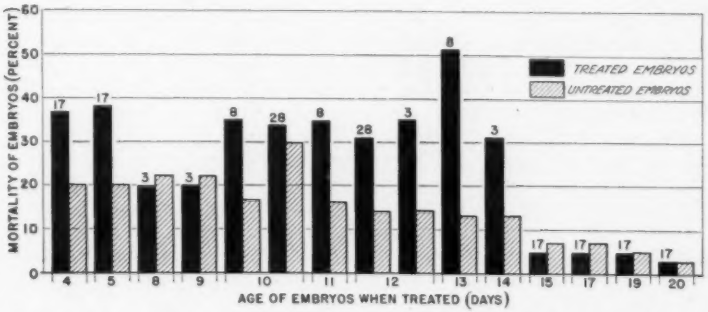

FIGURE 4.—Mortality among embryos in eggs subjected to shock from various sized charges of 20-percent gelatin dynamite. (Numbers at top of bars indicate number of half-pound sticks of dynamite used).

The eggs in the groups treated on the eighth, ninth, and fourteenth days of incubation were racked, large end up, in the incubator trays and the tray placed upon the surface of the ground. Directly over the eggs and within 1 foot of them three sticks of 20-percent dynamite were suspended and discharged. This was done to discover whether the shock transmitted through the air to the eggs was as severe as when transmitted through the ground.

After treatment all the eggs were carried back to the laboratory, where they were candled and those having broken shells were removed. The remainder were replaced in the incubator. The maximum time any group of eggs was out of the incubator during treatment was 30 minutes. The percentage of mortality of embryos in eggs that were not broken when subjected to this type of shock is shown in figure 4. The relative size of charges of dynamite used in each treated group is also indicated. Figure 4 shows the same general effect of this type of mechanical shock as was found in eggs which were shaken, jarred, or centrifuged. In the experiment with dynamite, however, the effect of the shock was cumulative and not so noticeable in mortality during the 72 hours following treatment as at the end of

the incubation period. The percentage mortality shown in figure 4, therefore, is the total mortality from time of treatment to end of incubation. However, this figure in general shows the same susceptible period to mechanical shock, with the exception of the 8- and 9-day points, as shown in the other treated groups, that is, from the fourth to the fifteenth day of incubation. Embryos in eggs subjected to shock on or after 15 days of incubation showed little or no effect due to treatment. The shock on the eighth, ninth, and fourteenth days, as previously pointed out, was transmitted to the eggs through the air instead of through the ground as in the case of the other treated groups. The charges of dynamite used to produce the shock at these points were smaller than those used on the other days of incubation. Either or both of these factors may help to explain the apparent discrepancy in the data.

SUMMARY AND CONCLUSIONS

The data presented show that chicken embryos can be killed by mechanical disturbances caused by jarring, centrifuging, or concussion of the air or ground. In addition, it is shown that embryos are more affected by mechanical shock from the fourth to the fifteenth day than at any other period during incubation. Mortality among embryos was caused chiefly by broken yolk sacs and ruptured blood vessels. In many cases, tremulous air cells were produced by the different treatments, especially when the force was applied at the blunt end of the egg, and in such cases mortality was heavy. The tremulous air cells, in eggs where the embryos survived the treatment, became fixed when the allantois attached again to the shell membrane.

From the results of this experiment, it is recommended that hatching eggs during the period from the fourth to fifteenth day be handled as gently as possible and that they be candled on or after the fifteenth day where this is practicable.

THE VITAMIN A VALUE OF COLOSTRUM AND MILK OF FOUR COWS DETERMINED BY THE SINGLE-FEEDING METHOD[1]

By M. M. KRAMER, *associate in food economics and nutrition*, MARGARET D. BAIR, *graduate assistant*, BERNICE L. KUNERTH, *nutrition technician*, and W. H. RIDDELL, *associate dairy husbandman, Kansas Agricultural Experiment Station*

INTRODUCTION

Some years ago colostrum was shown (*2*)[2] to be higher in vitamin A value than milk produced later in lactation. More recently Semb, Baumann, and Steenbock (*5*), using physical-chemical methods, have reported high vitamin A values for colostral butter. Dann (*1*) studied samples from 14 Milking Shorthorn cows, using colorimetric methods and, for the richest samples, biological tests also. He concluded that the colostrum may be 10 to 100 times as rich in vitamin A as later milk from the same cow.

The physical-chemical methods of determining vitamin A and a precursor, β-carotene, which have been developed in recent years, are useful and handy, but the biological methods remain fundamental, as they summarize total vitamin A activity of the vitamin itself and of any precursor or precursors (*6*).

PROCEDURE

Colostrum and milk samples for these experiments were obtained from four cows in the experiment station herd, fed the regular dairy herd ration, which consisted of a fair quality of alfalfa hay (about No. 2 grade), Atlas sorgo silage, and a grain mixture of yellow corn, bran, and cottonseed meal. Two of the cows freshened in late winter and received only the regular ration; the other two freshened in the spring and had access to rye pasture in April and May, for 40 and 58 days, respectively, before freshening (table 1).

Samples for the determinations were collected four times during the first week of lactation, at the end of the second week, and near the end of the fourth week. In this paper the collections made during the first two days were considered as colostrum. Each sample, representing a 24-hour composite, was stored and kept in a frozen condition until fed.

The single-feeding method of Sherman and Todhunter (*7*) was used for biological assay. Young rats of Wistar Institute strain, weighing 38 to 40 grams, were depleted of vitamin A reserves and divided into groups of 10 or more, comparable in weight, sex, and litter. Single feedings of colostrum or milk were given as indicated in table 2 and negative control rats were maintained throughout. Carotene (*4*) (International standard for vitamin A secured through the Bureau of Chemistry and Soils, United States Department of Agri-

[1] Received for publication July 7, 1937; issued March, 1938. Contribution No. 69, Department of Home Economics, and No. 113, Department of Dairy Husbandry.

[2] Reference is made by number (italic) to Literature Cited, p. 232.

culture) was dissolved in an edible cottonseed oil and stored in the dark below 0° C. until needed. Portions of this solution, equivalent to 10 micrograms, 17 micrograms, and 34 micrograms of pure β-carotene, were given as single feedings to the positive control animals to provide data for carotene reference curves (fig. 1).

TABLE 1.—*Description, lactation data, and composition of colostrum and milk of cows used in experiments*

Ration, cow number, and description	Day of lactation	Total daily yield of—			Milk content	
		Milk	Fat	Total solids	Fat	Total solids
		Pounds	*Pounds*	*Pounds*	*Percent*	*Percent*
Standard dairy ration:	First	9.5	0.36	1.64	3.8	7.27
	Second	7.4	.29	.94	3.9	12.76
190, 7 years old, fifth calf Feb. 3, 1935, Holstein.	Fourth	37.6	1.84	5.23	4.9	13.91
	Seventh	42.2	2.41	6.14	5.7	14.54
	Fourteenth	47.9	5.25	6.26	4.7	13.06
	Twenty-sixth	44.0	2.02	5.38	4.6	12.23
	First	8.0	.41	1.82	5.1	22.76
	Second	13.9	1.24	3.02	9.0	21.92
241, 3 years old, second calf Mar. 4, 1935, Ayrshire.	Fourth	28.5	1.36	4.29	4.8	15.05
	Seventh	31.5	1.51	4.54	4.8	14.41
	Fourteenth	32.5	1.40	4.32	4.3	13.28
	Twenty-fifth	34.6	1.45	4.53	4.2	13.01
Standard dairy ration plus rye pasture:	First	11.0	.37	2.49	3.4	22.66
	Second	32.0	1.63	6.39	5.1	19.98
220, 7 years old, fifth calf May 8, 1935, Ayrshire.	Sixth	54.7	2.63	9.24	4.8	16.89
	Twelfth	55.8	2.79	7.92	5.0	14.19
	Twenty-fifth	59.2	2.84	7.56	4.8	12.77
	First	5.2	.27	1.05	5.2	20.11
	Second	18.5	.94	2.84	5.1	15.37
	Fourth	31.6	1.42	4.33	4.5	13.69
471, 6 years old, fourth calf May 26, 1935, Guernsey.	Ninth	32.8	1.74	4.89	5.3	14.90
	Sixteenth	33.4	1.57	4.56	4.7	13.65
	Twenty-sixth	32.8	1.38	4.21	4.2	12.84

None of the cows used was a first-lactation heifer (table 1). Dann (*1*) stated that colostrum from heifers tended to be richer in vitamin A than colostrum from cows, and he suggested that the difference may have been due to the fact that the vitamin A reserve of the heifer had not been diminished by previous lactation. The yields of colostrum presented in table 1 are probably low because complete milking before the fourth day is frequently difficult and undesirable; hence the figures given do not represent entire production. Beginning with the fourth day, however, the yields are complete.

EXPERIMENTAL DATA

In table 2 are presented data from biological assay, summarized and checked against the International standard β-carotene. In each group 10 or more rats were started but it was necessary to eliminate some from the summary; for example, the bloody colostrum of cow 220, first and second days, seemed distasteful to the rats and many refused to eat their portions. The sex ratios of the groups, however, remained well balanced. The feeding experiments extended through about 9 months and included rats whose depleted weights varied from

80 to 105 g, the average for the groups ranging from 89 to 96 grams. The areas under the composite curves (fig. 1) have been shown by the originators of the method (7) to be directly proportional to the vitamin A value of the single feeding. The results of feeding carotene at three levels as indicated in table 2 show that this ratio of areas held for animals of the present series. Readings from the three carotene

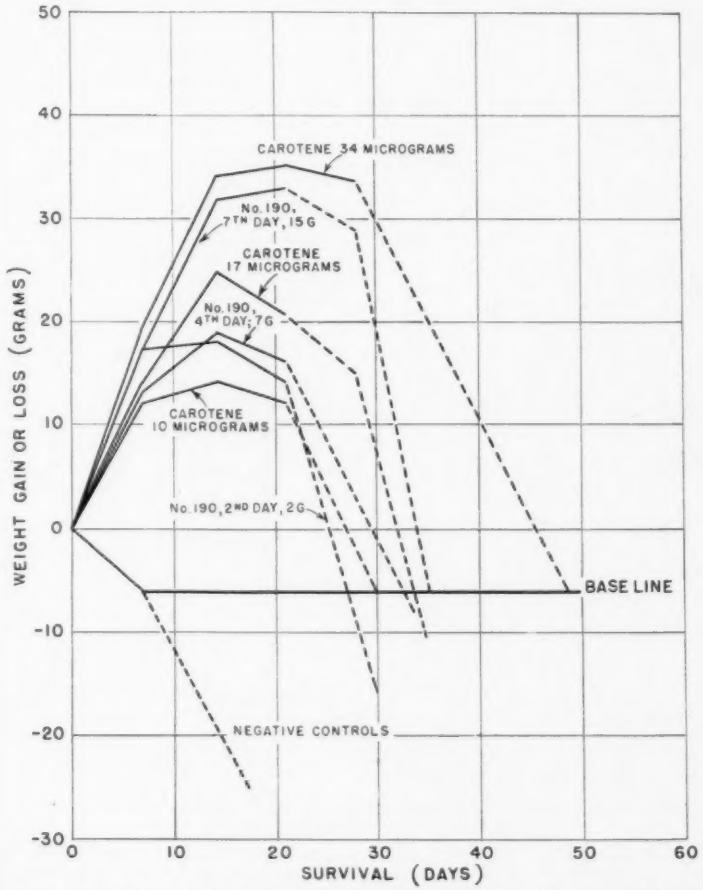

FIGURE 1.—Average gain or loss of rats which had been depleted of body stores of vitamin A and then given a single feeding of colostrum or milk from cow 190 or of carotene in quantities indicated.

reference curves indicate that 2.47 micrograms of pure β-carotene (equivalent to 4.10 International Units) was responsible for an area of 1 square inch. These figures were used to calculate the vitamin A value of 1 g. of each sample in terms of both β-carotene and International Units.

TABLE 2.—*Vitamin A values of colostrum and milk determined by the single-feeding method*

Source of sample		Yield per day		Rats			Figures from composite growth curves			
							Area		Equivalent value per gram in terms of—	
Cow number and ration	Day of lactation [1]	Colostrum or milk	Vitamin A value	Number	Average depleted weight	Single feeding	Total	Per gram fed	β-carotene	Vitamin A
		Grams	*1,000 International Units*		*Grams*	*Grams*	*Square inches*	*Square inches*	*Micrograms*	*International Units*
190 (no pasture supplement).	First [1]	4,309	70	10	90	2	7.90	3.95	9.76	16.20
	Second	3,357	30	7	90	2	4.38	2.19	5.41	8.98
	Fourth	17,055	49	8	91	7	4.90	.70	1.73	2.87
	Seventh	19,142	49	6	93	15	9.28	.62	1.53	2.54
	Fourteenth	21,729	44	8	90	20	9.80	.49	1.21	2.01
	Twenty-sixth	19,958	46	8	91	20	11.16	.56	1.38	2.29
241 (no pasture supplement).	First [1]	3,629	66	8	96	2	8.85	4.43	10.94	18.16
	Second	6,260	128	9	92	2	9.96	4.98	12.30	20.42
	Fourth	12,928	53	10	93	7	6.90	.99	2.45	4.07
	Seventh	14,288	40	7	94	15	10.17	.68	1.68	2.79
	Fourteenth	14,742	25	6	90	20	8.23	.41	1.01	1.68
	Twenty-fifth	15,695	32	5	89	20	10.00	.50	1.24	2.06
220 (supplement of rye pasture).	First [1]	4,980	127	9	96	2	12.40	6.20	15.31	25.41
	Second	14,515	178	6	96	2	5.98	2.99	7.39	12.27
	Sixth	24,812	48	7	92	7	3.28	.47	1.16	1.93
	Twelfth	25,311	46	6	95	15	6.53	.44	1.09	1.81
	Twenty-fifth	26,853	33	7	90	20	5.92	.30	.74	1.23
471 (supplement of rye pasture).	First [1]	2,359	65	8	94	2	13.43	6.72	16.60	27.56
	Second	8,392	162	10	92	2	9.39	4.70	11.61	19.27
	Fourth	14,334	62	10	93	7	7.42	1.06	2.62	4.35
	Ninth	14,878	35	8	91	15	8.60	.57	1.41	2.34
	Sixteenth	15,150	28	9	90	20	8.93	.45	1.11	1.84
	Twenty-sixth	14,878	25	5	90	20	8.25	.41	1.01	1.68
Controls:						*Micrograms*				
β-carotene				11	91	10	[2] 4.14			
				23	92	17	[3] 6.73			
Negative [4]				9	92	34	[4] 13.67			
				25	93					

[1] The first sample in each case was collected on the day of freshening.
[2] 1 square inch = 2.41 micrograms of pure β-carotene.
[3] 1 square inch = 2.48 micrograms of pure β-carotene.
[4] 1 square inch = 2.53 micrograms of pure β-carotene.

Average = 2.47 micrograms of pure β-carotene = 4.10 International Units.

When comparisons were made on the basis of the vitamin A value per gram (table 2) the secretions of the first or second day of lactation were highest. The two cows receiving pasture supplement produced the richest samples of colostrum tested, containing 25 and 28 International Units of vitamin A per gram. This is consistent with accepted findings (*3*). The vitamin A value per gram of sample dropped rapidly during the first days of lactation (fig. 2, *B*, *D*), and by the end of the first week approached the level maintained during the remainder of the first month. The first samples secured from these two cows were 16 to 20 times as potent as the normal milk at the end of the month.

The richest samples secured from the two cows on the unsupplemented ration contained 16 to 20 International Units per gram. As in the other cases, the vitamin A value decreased rapidly (fig. 2, *A*, *C*). After the first week these cows produced milk containing about 2 International Units per gram. Sherman (*6*) summarized data from many sources and reported the vitamin A value of cow's milk to be 2.92 ± 0.12 International Units per gram.

The rapid drop in vitamin A value per gram of sample as lactation progressed was paralleled by the drop in vitamin A value of the total yield per cow per day (fig. 2). The low vitamin A value for the total yield of cow 190 on the second day of lactation was probably due to

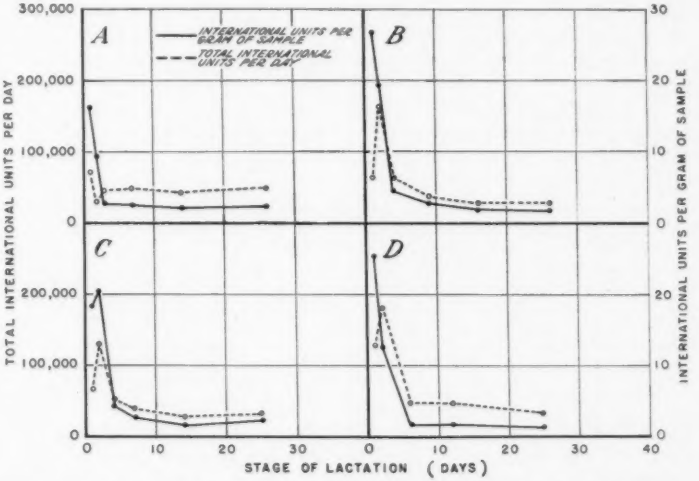

FIGURE 2.—Vitamin A value of colostrum and of milk per gram and per day: *A*, Cow 190, Holstein, no pasture supplement; *B*, cow 471, Guernsey, rye pasture supplement; *C*, cow 241, Ayrshire, no pasture supplement; *D*, cow 220, Ayrshire, rye pasture supplement.

difficulty in obtaining colostrum. Toward the end of the first week, the curve of vitamin A yield per day flattened out for each cow (fig. 2). It is interesting to note that cow 190, a Holstein receiving no pasture, maintained a daily vitamin A output higher than that of any of the other cows.

SUMMARY AND CONCLUSIONS

Samples of colostrum and milk were collected at intervals during the first month of lactation from two cows on the regular dairy herd ration and from two cows on this ration supplemented with rye pasture. The total vitamin A values of the samples were determined by the single-feeding biological method. Reference curves were secured by feeding standard carotene to positive control rats.

The secretions of the first or second day were richest in total vitamin A value, 25 and 28 International Units per gram for the cows receiving rye pasture supplement and 16 and 20 International Units for the others. By the fourth day of lactation there was a considerable drop

in the vitamin A value per gram of sample. In general the vitamin A value of the milk remained much the same from the seventh day to the end of the experiment, approximately 2 International Units per gram. It is interesting to note that one of the cows that received no rye pasture supplement maintained the highest level of vitamin A value per gram of milk throughout the month.

When comparisons are made on the basis of total production of vitamin A per day, the largest total output was found on the first or second day of lactation. Again the cows that received the rye pasture supplement exceeded those that did not, the maximum production of the former being 178,000 and 162,000 International Units and that of the latter 70,000 and 128,000. The rapid drop in vitamin A value per gram of sample as lactation progressed was paralleled by the drop in vitamin A value of the total yield per cow per day. One cow receiving no supplement maintained the highest level of vitamin output during the latter part of the month. In general, the vitamin A value of the total daily production was near the same level from the end of the first week until the end of the month.

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VITAMIN G (RIBOFLAVIN) CONTENT OF COLOSTRUM AND MILK OF COWS DETERMINED BIOLOGICALLY[1]

By M. M. KRAMER, *associate in food economics and nutrition*, INEZ GARDNER, *graduate assistant*, BERNICE L. KUNERTH, *nutrition technician*, and W. H. RIDDELL, *associate dairy husbandman, Kansas Agricultural Experiment Station*

INTRODUCTION

Cow's milk is recognized as a valuable source of vitamin G, but the influence of the individuality of the cow and the stage of lactation on the vitamin G content of milk has not been established. This study deals with the vitamin G (riboflavin) content of milk with special reference to the influence of these two factors.

PROCEDURE

Twenty-four-hour composite samples of colostrum and milk were obtained from four cows (table 1) that received the regular dairy herd winter ration, consisting of a fair quality of alfalfa hay (about No. 2 grade), Atlas sorgo silage, and a grain mixture of yellow corn, bran, and cottonseed meal. All the cows in the experiment freshened in the fall and winter and had no access to pasture in the period immediately preceding or during the collection of samples. The samples were stored at a temperature below 0° C. until used, as Grayson (*6*) [2] has shown that there is no deterioration in the vitamin G of milk during storage if the milk is kept frozen. The first samples (series 1), collected on the first or freshening day and at the end of the first month of lactation gave results indicating superiority of colostrum in vitamin G content. Series 2 was then planned, with an additional collection on the fifth day, to show rate of decline in vitamin G potency of milk. Previous studies in this laboratory had indicated that the vitamin A value of milk at the fifth-day level is approaching normal.

TABLE 1.—*Description, lactation data, and composition of colostrum and milk of cows used in experiments*

Source of sample		Total daily yield of—			Milk content	
Cow no. and description	Day of lactation	Milk	Fat	Solids	Fat	Total solids
		Pounds	*Pounds*	*Pounds*	*Percent*	*Percent*
118, 3 years old, second calf, Holstein.	First	7.0	0.29	0.95	2.9	13.50
	Thirtieth	46.2	1.74	5.51	3.8	11.93
373, 4 years old, third calf, Jersey	First	6.0		1.08		17.92
	Thirtieth	30.1	1.92	4.69	6.4	15.59
197, 6 years old, fourth calf, Holstein.	First	4.4	.24	1.15	5.4	26.17
	Fifth	47.0	2.51	6.81	5.3	14.49
	Thirtieth	63.5	2.82	8.17	4.4	12.87
378, 3 years old, second calf, Jersey	First	6.5	.27	1.13	4.2	17.32
	Fifth	23.9	1.53	3.79	6.4	15.87
	Thirtieth	28.8	1.83	4.77	6.7	16.55

[1] Received for publication July 7, 1937; issued March, 1938. Contribution No. 70, Department of Home Economics, and No. 114, Department of Dairy Husbandry.

[2] Reference is made by number (*italic*) to Literature Cited, p. 237.

The biological method of Bourquin and Sherman (*3*) was used for vitamin G (riboflavin) assay. Extensive investigation of the components of vitamin G (B_2) has aroused concern regarding the validity of methods of biological assay, but Booher, Blodgett, and Page (*2*) and, more recently, Bisbey and Sherman (*1*) have confirmed the accuracy of the Bourquin-Sherman (*3*) method. All rats (Wistar Institute strain) were kept in cages with raised screen floors and checked daily so that no animal consuming excreta would be retained on experiment. The rats of series 1, having been kept on stock diet until 4 weeks of age (*3*), were larger and older than those of series 2. The rats of series 2 were fed the stock diet only until they had reached weights of 38 to 40 grams; this procedure was in accordance with the suggestion of Roscoe (*10*), who used 30- to 40-gram rats, Supplee et al. (*11*), who used animals 20 to 26 days old and preferably 40 to 45 grams in weight, and Prunty and Roscoe (*9*), who used rats weighing 35 to 50 g. Lassen (*7*) also preferred 3 weeks to 4 weeks old rats as they were more uniform in size and varied less in subsequent response. Depleted weights for rats of series 2 were correspondingly lower. Samples to be assayed were fed in daily portions as indicated in table 2. Riboflavin [3] (lactoflavin, PX grade) was fed as a standard for reference, and herd milk was given to another group of rats for comparison. Negative controls were maintained throughout.

TABLE 2.—*Vitamin G (riboflavin) content of colostrum and milk determined biologically*

Source of sample		Yield of milk per day	Rats					Estimated flavin—		Yield of vitamin G per day
Series and cow number	Day of lactation		Number	Average initial weight	Average weight when depleted	Average gain in 8 weeks	Amount of sample fed	Per feeding	Per gram	
										1,000 micrograms
Series 1:		*Grams*		*Grams*	*Grams*	*Grams*	*Grams*	*Micrograms*	*Micrograms*	
118	1st	3,175	9	59	68	30	1	4.5	4.5	14
	30th	20,956	10	56	63	33	3	5.0	1.7	36
373	1st	2,722	10	57	62	30	1	4.5	4.5	12
	30th	13,653	10	51	62	42	3	6.4	2.1	29
Series 2:										
197	1st	1,996	11	39	47	44	1	6.7	6.7	13
	5th	21,319	11	39	50	49	2	7.4	3.7	79
	30th	28,803	10	38	46	47	3	7.1	2.4	69
378	1st	2,948	11	38	48	28	1	4.2	4.2	12
	5th	10,841	11	39	46	43	2	6.5	3.3	36
	30th	13,063	11	39	48	46	3	7.0	2.3	30
Controls:										
Herd milk, winter		.....	14	39	45	40	3	6.0	2.0	.....
Lactoflavin (Labco PX).		.....	9	39	49	35	[1] 5.0	.....	.....	.....
Negative controls		.....	25	39	46	±0	.....	.....	.....	.....

[1] Micrograms.

EXPERIMENTAL DATA

At present it seems desirable to estimate vitamin G as micrograms of riboflavin (table 2) by comparison with the flavin reference curve.

[3] Secured from Bordon Co.

No flattening of this curve occurred to suggest an unknown limiting factor in the vitamin G-free diet.

Judging by the composite growth curves of the rats (fig. 1), 1 g of first day colostrum, 2 g of fifth-day, and 3 g of thirtieth-day milk contained comparable amounts of vitamin G. The richest sample studied was the colostrum produced by cow 197, containing 6.7 micrograms per gram, while the other colostral samples had estimated values of 4.2 and 4.5 micrograms per gram (table 2, fig. 2). The two fifth-day samples were less potent than colostrum produced by the same cows, containing only 3.7 and 3.3 micrograms of flavin per gram. Differences in vitamin G content of samples due to stage of lactation

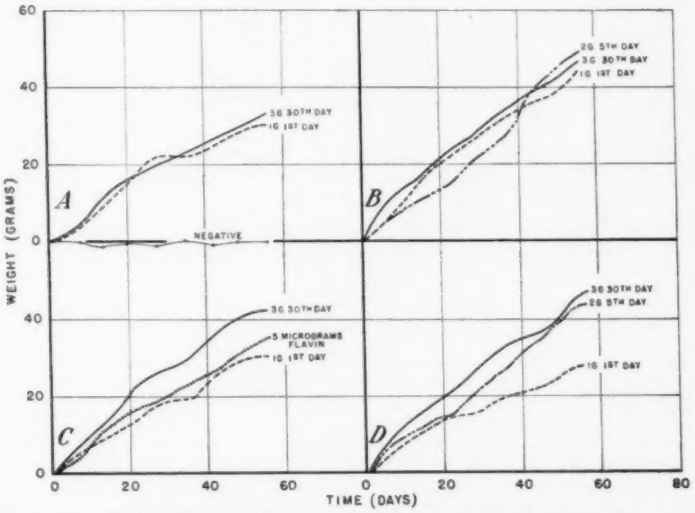

FIGURE 1.—Gain in weight of rats fed colostrum or milk: *A*, From cow No. 118, series 1; *B*, from cow No. 197, series 2; *C*, from cow No. 373, series 1; *D*, from cow No. 378, series 2.

are more pronounced than differences between samples of the four individual cows at the same stage of lactation.

Milk of the thirtieth day was still less potent, containing 1.7 to 2.4 micrograms of flavin per gram, with an average of 2.1, which is similar to 2.0 micrograms, the estimated value of herd milk. The vitamin G content of herd milk was, therefore, about 1,950 micrograms per quart. Other work (*5*) indicates that the flavin content of milk may be 2 to 3 micrograms per milliliter. MacLeod et al. (*8*) concluded that milk from the Walker-Gordon dairy contained 0.3 of a Bourquin-Sherman unit of vitamin G per gram. Day and Darby (*4*) have suggested that a Bourquin-Sherman unit equals 4 micrograms of lactoflavin, a ratio consistent with findings in this laboratory. On this basis, the Walker-Gordon milk contained 1.3 micrograms per gram, while pasteurized milk available in New York City contained 3 micrograms per gram, according to Todhunter (*12*).

Total 24-hour yields of vitamin G for each of the four cows used in the investigation were estimated for the stages of lactation studied

and recorded as thousands of micrograms. The yields of vitamin G from the four cows for the first day of lactation were similar, 12,000 to 14,000 micrograms. These values are probably low because of the inadvisability of complete milking. Data from only two cows were available for the fifth day, and these showed a marked difference (fig. 2). However, it is interesting to note that each cow produced somewhat more vitamin G on the fifth day than on the thirtieth day, with cow 197 higher each time. This cow, a Holstein, excelled in other ways. At the end of the first month of lactation, when she was yielding more than twice as much vitamin G (69,000 micrograms) per day as the mean production of the other cows (32,000 micrograms), she also produced 81 percent more milk, 51 percent more butterfat,

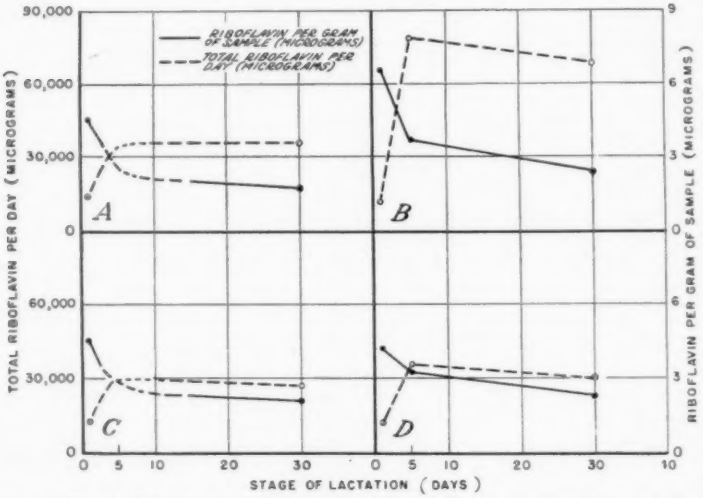

FIGURE 2.—Vitamin G (riboflavin) per gram and per daily yield of colostrum and milk: *A*, Cow No. 118 series 1; *B*, cow No. 197, series 2; *C*, cow No. 373, series 1; *D*, cow No. 378, series 2.

and 43 percent more total solids than the other cows. In contrast to the drop in vitamin G content per gram of milk as lactation progressed, the total daily output of the vitamin per cow increased owing to the increase in the yield of milk. The relationship of content per gram to total yield of flavin is presented graphically in figure 2.

SUMMARY AND CONCLUSIONS

Twenty-four-hour samples of colostrum and milk from two Jersey and two Holstein cows on the first, fifth, and thirtieth days of lactation, together with a sample of herd milk, were tested for vitamin G (riboflavin) content by biological assay. Flavin reference curves were used to estimate the vitamin G content per gram of sample. The vitamin G content of milk from individual cows at the end of 1 month of lactation and also a composite herd sample was estimated as about 2 micrograms per gram, or 1,950 micrograms per quart.

The samples of fifth day milk tested were approximately 50 percent richer in vitamin G than the later milk from the same cows or the regular herd milk. First-day colostrum was richest in vitamin G and was estimated to contain 4.2 to 6.7 micrograms per gram. A gram of colostrum, 2 g of fifth-day milk, and 3 g of milk at the end of the first month of lactation gave approximately the same total gains in weight for the groups of rats fed, indicating a similar vitamin G content.

On the first day of lactation the cows all yielded somewhat more than 12,000 micrograms of vitamin G. The largest total daily outputs were on the fifth day for the cows from which such samples were saved, but these totals approached the yields for the thirtieth day. On the thirtieth day of lactation three cows produced about 32,000 micrograms each, while the other cow, a high milk producer, yielded twice this amount of vitamin G.

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LIFE SPAN AND MORPHOLOGY OF FIRE BLIGHT BACTERIA AS INFLUENCED BY RELATIVE HUMIDITY, TEMPERATURE, AND NUTRITION[1]

By H. R. ROSEN

Professor of plant pathology, Arkansas Agricultural Experiment Station

INTRODUCTION

The problem concerning the sources of inoculum for the earliest or primary fire blight on apple and pear trees in the Ozarks of Arkansas remains obscure. Unlike more northern and western fruit-growing areas, where excellent evidence has been found for the overwintering of the pathogen in formerly blighted limbs and twigs and its dissemination from such hold-over blight to blossoms and twigs, no such evidence has been found in the Ozarks (*11, 13, 14*).[2] This should occasion no surprise. The marked differences in climatic conditions, in length of growing season, and in varieties grown must undoubtedly have some influence on the variation of the life of the pathogen. The relative scarcity or absence of fire blight west of the Cascades is well known, in contrast to its prevalence in fruit-growing areas east of this mountain range, and as Heald (*7*) has noted:

> * * * sufficient time has elapsed since its arrival in the coast country for it to have become prevalent in western Washington, hence it seems possible that climatic factors are responsible for its absence from this section.

In an effort to discover the sources of inoculum for early spring infections in the Ozark region, various lines of investigation have been pursued, including the possible overwintering of fire blight bacteria within beehives (*13*) and the overwintering of bacterial exudate either on parts of the trees above ground or in the soil (*16*). However, none of the evidence deduced from these studies is conclusive (*13, 16*). The problem is still unsolved, not only for the Ozarks of Arkansas but for the Southern States as a whole, although the use of open-blossom sprays by the writer (*14*) has been based on the assumption that the first spring blight is occasioned mainly by the dissemination of the bacteria by blossom-visiting insects, especially bees.

It is clear, however, that without knowledge of the influence exerted by various combinations of climatic and nutritional factors on the life of this parasite, conclusions relative to the longevity and prevalence of bacteria drawn from such tests as artificial contamination of beehives, attempted isolations from infested hives, and isolations from insect bodies, soil, or diseased tree parts may have very restricted significance and at best be applicable only to local or purely artificial conditions. To gain more definite information on the influence of

[1] Received for publication July 27, 1937; issued April 1938. Research paper No. 483, of the Journal Series, University of Arkansas.

[2] Reference is made by number (italic) to Literature Cited, p. 257.

important climatic factors and to a limited extent of nutritional factors on the life of the organism, investigations reported here were undertaken. A special effort was made to study the behavior of the bacteria in naturally occurring exudate rather than their behavior in pure, artificial cultures, although the latter were used for comparison.

REVIEW OF PREVIOUS WORK

Since a review of the older literature pertaining to the influence of dryness on the longevity of the fire blight organism, *Erwinia amylovora* (Burrill) Winslow et al. (*Bacillus amylovorus*), has been presented previously (*16*), there is no need of duplicating it here. In that paper evidence was presented to show that the bacteria in the form of exudate derived from blighted tissues are very much less sensitive to dry air than had generally been supposed; and that at a controlled relative humidity approaching zero they were found to be alive at the end of a year under laboratory conditions of fluctuating temperatures and reduced light. Likewise, it was reported that exudate kept outdoors in corked vials, under uncontrolled light, temperature, and humidity conditions, yielded dead bacteria in short order, and that the organisms in the form of hardened exudate left in place on a cankered pear trunk exposed to direct sunlight also died within a few days.

Good evidence has very recently been presented by Parker (*10*) confirming the findings relative to the longevity of *Erwinia amylovora* under dry conditions, and adding to our knowledge concerning the combined influence of temperature and humidity. Using temperatures of 31°, 52°, and 70° F. and both very dry conditions, maintained by the use of calcium chloride desiccators, and more moist conditions in open dishes, he found that bacteria derived from exudate lived at least 52 days, the last day of testing, at 31° and 52°, and at least 45 days at 70°, all under dry conditions, while under more moist conditions in open dishes they were shorter lived.

Ark (*1*) has also reported very recently on studies of fire blight bacteria which included the influence of dryness on the life of the bacteria. First he reports very briefly that he "was able to isolate viable bacteria from bacterial exudate kept in a vial in a dry laboratory closet for 2 years and 10 months." Whether these bacteria were tested for pathogenicity is not stated, and one wonders what tests were used to determine if the isolated bacteria actually represented *Erwinia amylovora*. However, he performed two additional experiments involving the influence of dryness, though both were primarily concerned with discovering differences in reaction of different isolates. Using four isolates derived from unlike hosts and from diverse parts of the United States, he grew them in beef-extract agar, suspended the growths in a saline solution, and tested for the influence of dryness by smearing a drop of the bacterial suspensions on cover glasses maintained at different degrees of dryness. The bacteria survived from 18 to 36 hours, the latter period representing the longest utilized. "The degree of humidity seemed to be of no significance as far as the longevity of *E. amylovora* is concerned in this experiment," he concluded. In a second experiment, which also involved artificial culture media, gauze strips suspended in broth cultures were removed after 48 hours of growth, the strips hung in large sterile test tubes, and pieces cut off and cultured from time to time. Two isolates were

found to be nonviable after 6 days, a third after 4 days, and the fourth after 10 days. He reported:

> Both of the experiments were repeated and in each case the same results were obtained. Thus, it is safe to conclude that different degrees of susceptibility to dryness exist among isolates of *Erwinia amylovora* * * *.

Ark makes no comment of the amazing discrepancy in longevity under dry conditions shown in his test of viability of bacterial exudate compared with his tests of pure cultures.

So far as studies on the influence of temperature are concerned, most of these have in the past involved pure cultures, usually maintained in beef-broth or synthetic media in which the standardized procedure of determining maximum, optimum, and minimum temperatures for growth was followed and little attention paid to the life span of the bacteria at the different temperatures. With the exception of the very recent paper by Ark (*1*), these former temperature studies have been reviewed (*14, p. 4*). Ark followed the same procedure used by previous investigators and obtained comparable results.

With reference to nutritional factors influencing the longevity of this organism, the writer has no knowledge of earlier work. At times it has been assumed that fire blight bacteria grown in beef-broth or nutrient agar will be comparable, morphologically and physiologically, to those growing within apple or pear tissues, and such tests as applying beef-broth or agar cultures to beehives followed by attempts to reisolate the parasite after varying intervals of time from the hive have been used with the assumption that the data obtained would be comparable to those resulting from the transfer of bacteria from a pear or apple blossom to the hive by a honeybee. That differences in nutrition—as exemplified by growth in beef-broth or nutrient agar compared with bacterial growth in natural host tissues, or in nectar—might conceivably yield wholly unlike results in physiological response as well as in morphology had not, to the writer's knowledge, been entertained.

Since part of the investigations herein described concerns differences in morphological response, particularly that which deals with the presence or absence of a capsule, sheath, or slime formation, several investigators report the absence of capsules for this species of bacteria (*5, 8, 19*).

MATERIALS AND METHODS

The bacterial exudate utilized consisted of four collections. Lot 1 was made up of the exudate placed over concentrated sulphuric acid on June 11, 1934, used in earlier work (*16*) and continued in these investigations.

Lot 2 consisted of exudate gathered on May 21 and 28, 1935, from freshly blighted shoots and blossom clusters of Jonathan apple trees located in a commercial orchard near Rogers, Ark. This material was kept in a refrigerator overnight and placed the next day in small, paraffin-covered paper baskets suspended in 200-cc bottles over concentrated sulphuric acid having a specific gravity of 1.835. Most of this exudate when gathered was in the form of small, milky, or light-brown droplets which had not fully hardened and were still quite viscid. In many instances it was found necessary to scrape the epidermal hairs of the young twig, petiole, or pedicel, so that the droplets of exudate as placed in the bottles were often enmeshed in these hairs. No attempt was made to sterilize the bottles or to remove the